Human leukocyte antigen (HLA) diversity and clinical applications in South Africa

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The major histocompatibility complex, known as the human leukocyte antigen (HLA) complex in humans, forms an integral component of adaptive T cell immunity by presenting self and non-self peptides to the T cell receptor, thereby allowing clonal expansion of responding peptide-specific CD4^{+} and CD8^{+} T cells. HLA likewise forms an integral part of the innate immune response through the binding of killer-cell immunoglobulin-like receptor (KIR) molecules, which regulate the response of natural killer (NK) cells. The HLA complex is found on the short arm of chromosome 6 and is the most polymorphic region in the human genome. Africans are genetically more diverse than other populations; however, information on HLA diversity among southern Africans, including South African populations, is limited. Paucity of African HLA data limits our understanding of disease associations, the ability to identify donor-recipient matches for transplantation and the development of disease-specific vaccines. This review discusses the importance of HLA in the clinical setting in South Africans and highlights how tools such as HLA imputation might augment standard HLA typing methods to increase our understanding of HLA diversity in our populations, which will better inform disease association studies, donor recruitment strategies into bone marrow registries and our understanding of human genetic diversity in South Africa.

The major histocompatibility complex (MHC), referred to as the human leukocyte antigen (HLA) complex in humans, is located on the short arm of chromosome 6 (Fig. 1). This region encodes cell surface proteins that form part of the innate and adaptive immune responses through binding killer-cell immunoglobulin-like receptor (KIR) molecules on the surfaces of natural killer (NK) cells and the recognition and binding of self and non-self peptides.

The HLA region is the most polymorphic region in the human genome.[1] Allelic variants mostly arise within the nine classic genes (*HLA-A, -B, -C, -DPA1, -DPB1, -DQA1, -DQB1, -DRA* and *-DRB1*) of the HLA region. Classic HLA molecules present peptides to the T cell receptor (TCR) of CD4^{+} and CD8^{+} T cells, while non-classic HLA molecules mediate inhibitory or activating stimuli.[2] There are currently 21 499 HLA alleles listed in the IMGT/HLA database (https://www.ebi.ac.uk/ipd/imgt/hla/stats.html release 3.35.0 January 2019), of which 15 586 are class I and 5 913 class II alleles (Fig. 2).[3]

The class I HLA region spans >2 000 kb and consists of ~20 genes. There are three classic HLA genes within the class I region: *HLA-A, -B,* and *-C.* The *HLA-B* locus is the most polymorphic of the class I genes,[4] with 5 881 alleles currently documented in the HLA database, while *HLA-A* has 4 846 and *HLA-C* 4 654 alleles.[3] The classic HLA class I genes consist of eight exons, while the polymorphisms reside in gene regions that encode the peptide-binding groove. Class I molecules consist of two chains, the α chain and non-covalently bound β$_2$-microglobulin. The α1 and α2 chains are the variable regions within the class I genes. These variable regions form the peptide-binding groove designed to bind endogenously derived peptides, which in turn are recognised by the TCR on CD8^{+} T cells. The peptide-binding groove binds endogenously derived peptides, which in turn are recognised by the TCR on CD8^{+} T cells. The class I endogenous pathway is associated with defence against intracellular
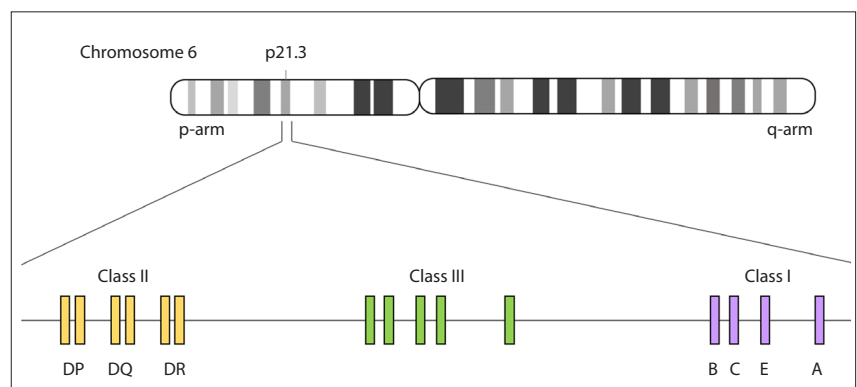

Fig. 1. The HLA region on chromosome 6. The HLA region is located on the short arm (p21.3) of chromosome 6 and spans >3.6 Mb. This region comprises three classes (I, II and III) that play an important role in immune responses.
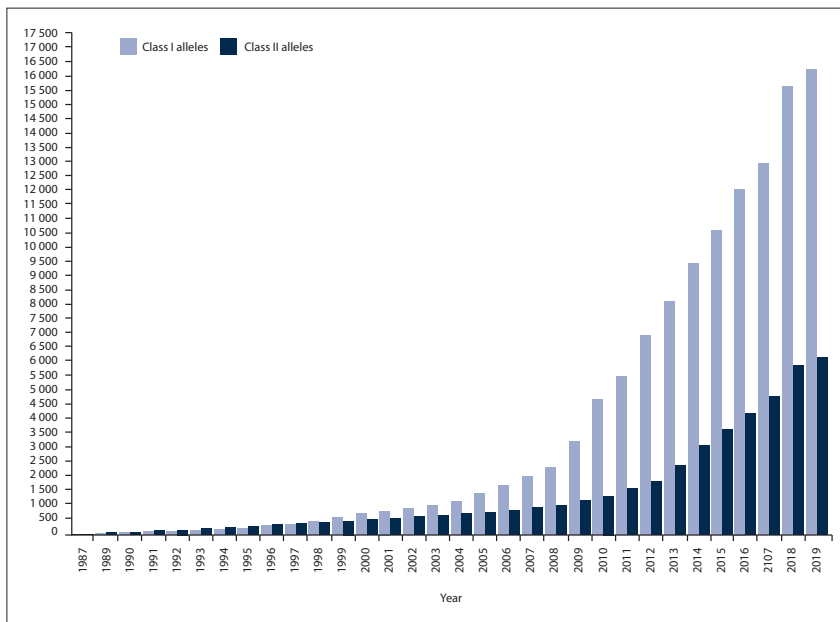
Fig. 2. The number of HLA alleles has been increasing since 1987 owing to advancement in typing methods. There are currently more than 14 000 and 5 000 class I and II alleles respectively in the IMGT HLA database (figure from http://hla.alleles.org/inc/images/graph_hires.png, accessed 23 April 2019).[3]

pathogens such as viruses. In addition, class I molecules are also recognised by KIRs which mediate tolerance and response of NK cells.

Class II molecules include the classic *HLA-D* genes, which are subdivided into DQ (*DQA1* and *DQB1*), DP (*DPA1* and *DPB1*), and DR (*DRA* and *DRB1*), and are restricted to immune competent cells (B cells, macrophages, and endothelial cells of T cells). The class II genes encode proteins that are expressed on the cell surface of antigen-presenting cells (APCs), where they present peptides to helper T cells. Within the class II genes, exon 2 is the most variable region and also forms the peptide-binding groove. All class II molecules consist of two transmembrane chains: α and β domains. The extracellular component of each class II molecule consists of two domains (α1, α2 and β1, β2). The α1 and β1 domains form the peptide-binding groove. Broadly, class II molecules are involved in the exogenous pathway and are associated with defence against extracellular pathogens such as bacteria. The TCR, which binds to the exogenously-derived peptide class II HLA complex, is found on CD4+ helper T cells.

The human immune system has evolved to interact with and mount immune responses against viral, parasitic, bacterial and other pathogen-derived peptides through high HLA diversity across populations. HLA needs to present an enormous array of antigenic peptides to T cells so that a unique immune response to a wide variety of peptides can be elicited.

The mechanisms that have been proposed to act on the evolution of HLA genes include: (*i*) accumulation of deleterious variants in nearby genes;[5] (*ii*) gene conversion/ interlocus genetic exchange;[6] (*iii*) over-dominant balancing selection (heterozygote advantage);[7] and (*iv*) frequency-dependent selection.[8] The HLA variability observed can also be the result of the presence of duplicated genes with similar or overlapping functions.[9] This assumption is made on the basis of the observation that the HLA complex consists of genes with similar but not completely identical structure and function. The variants within these genes mostly arise in the form of single-nucleotide polymorphisms (SNPs) and have directed the allelic diversity observed today.[10] The diversity of these molecules has occurred due to the presence of different alleles at a specific locus within a species. The alleles can differ from one another by an alteration at a minimum of one SNP. Several thousand allelic variants of the HLA genes have already been described, with many alleles being present at frequencies below 1%.[11] The variation observed is often population-specific and accounts for the diversity among populations.[12] The divergence rate of the HLA genes is due to the long history of independent haplotype evolution, where Africans (including South Africans) are considered to be genetically more diverse than other populations.[13] This has been shown using several genetic markers[14–16] including HLA genes.[17] Interestingly, most

HLA gene families that exist globally are found in African populations.[18]

The presence of specific HLA alleles in black South Africans has recently been reviewed by Tshabalala *et al.*[19] however, HLA typing data for these individuals remains limited. The under-representation of black South Africans in the South African Bone Marrow Registry (SABMR) accentuates the paucity of HLA typing data available for this population. There is therefore an increased need for HLA typing data in black South Africans.

HLA typing methods

HLA typing methods have evolved over time from phenotypic identity using serology to genotyping at high resolution using DNA sequencing technology. Serology-based methods identify HLA molecules to antigen level, with DNA methods being able to identify to protein level. Despite high resolution, sequencing-based typing (SBT) has limitations of usually typing certain exons within the HLA loci, thereby giving partial sequences of about 10% of the reported alleles.[20] Clinical HLA typing laboratories rarely sequence exons/ introns outside the peptide-binding groove for transplantation matching, with the assumption that they are not directly involved in T cell allo-recognition.[21,22] This assumption is supported by modelling HLA/ peptide/ TCR interactions,[23] and studying allele-specific peptide repertoires[24] and other allo-recognition studies.[25] Ambiguous allele combinations arising due to heterogeneity limit resolution in SBT, making it difficult to accurately assign HLA alleles. It is possible to sequence the entire HLA gene region using current SBT methods, but at a very high cost and requiring specific expertise. Another potential source of ambiguity in SBT is the *cis/trans* assignment of DNA bases in a heterozygous sample.[26] There have been advances in the use of next-generation sequencing (NGS) to improve coverage of HLA gene loci at high throughput, while at the same time reducing ambiguity associated with SBT.[27] Challenges of NGS HLA typing include the high number of polymorphisms associated with this gene region, with most individuals having heterozygous genotypes for most alleles. Other problems arise from the high number of pseudogenes in this region and long indels which cannot be efficiently covered by basic sequencing. The complex nature of some loci makes reference-based alignment of NGS reads less reliable.[28] To fully appreciate NGS HLA typing tools, there is a

need for complete full-length HLA allele sequences in the reference database.[27]

HLA imputation as a tool for better understanding HLA diversity

There is an information gap regarding the extent of HLA genetic diversity among South African populations and southern Africans in general.[19] Additionally, there are limited high-resolution HLA allele frequency data (except for HLA-disease associations) publicly available for the SA population.[11] Advances in computational biology make it possible to impute HLA alleles at a high degree of accuracy by inferring them from surrounding SNP markers across the MHC region[29] based on the high linkage disequilibrium (LD) within this region.[30] Additionally, high-resolution (up to 8 digit typing) HLA genotyping from whole genome sequence (WGS) and whole exome sequence (WES) datasets is possible from existing resources.[31–34] These tools borrow from existing genomic projects and studies to better understand HLA diversity in those populations. There are several efforts to understand genetic diversity in Africans and South African populations in general, including the Southern African Human Genome Programme (https://www.sahgp.org/index.php),[35] H3 Africa (https://h3africa.org/), 1000 Genomes project (http://www.internationalgenome.org/), and the African Genome Variation Project (https://www.sanger.ac.uk/science/collaboration/african-genome-variation-project). There are numerous other studies generating WGS, WES and SNP data from SA and African populations from which data for HLA imputation may be accessed. Several HLA imputation tools, including SNP2HLA,[36] HLA Genotype Imputation with Attribute Bagging (HIBAG)[37] and HLA*IMP[38] have been used to successfully determine HLA genotypes from SNP data. Despite the high imputation accuracy, these tools will augment, but not replace, routine HLA typing methods.

HLA applications

The role of HLA in the recognition of self and non-self peptides was first described 60 years ago. Since this discovery, there has been an increasing body of knowledge that emphasises the important role of HLA in basic and clinical immunology. The initial groundwork of the HLA complex was performed by Dausset[39] in 1958, and earned him a Nobel prize in 1980. His work was based on antibodies detected in multiparous women and multitransfused patients which reacted against leukocytes of numerous, but not all, individuals. These alloantibodies were proposed to play a critical role in tissue transplantation. A primary application of HLA typing is donor-recipient matching for solid organ and haematopoietic stem cell transplantation (HSCT). HLA mismatching between a haematopoietic stem cell donor and a recipient could result in a higher risk of rejection and occurrence of graft vs. host disease (GVHD) in the transplanted recipient. Four-digit molecular typing and a minimum of 9/10 matched alleles at five HLA loci (HLA-A, -B, -C, -DQB1 and -DRB1) between donor and recipient are imperative to ensure engraftment success following a bone marrow-derived HSCT.[40] The degree of HLA matching between donor and recipient varies with the type of transplantation; solid-organ transplantation requires less stringent HLA matching than does HSCT. In South Africa, for example, HLA-A, -B, -C and -DR typing for renal allograft donor-recipient matching is not done at all centres, and is done more to precisely identify donor-specific antibodies (DSAs) to the kidney allograft. Pre-transplant DSAs would be a contraindication to a transplant, for example, or would indicate tailoring post-transplant immunosuppressive treatments. The increased longevity of grafts

transplanted in accordance with lower HLA mismatched loci is well-known[41] and has been confirmed in a meta-analysis of over 480 000 transplanted patients.[42] HLA typing and consequent matching of HLA donor-to-recipient phenotype forms an integral part of the bone marrow graft allocation protocol internationally as well as in the SA transplantation fraternity. Among other criteria for patients awaiting transplantation, novel strategies are being employed to establish improved HLA matching between unrelated living donors and recipients.

Most studies focus on the immuno-regulatory role of HLA and its related diagnostic or disease-associated applications. HLA disease associations have been published from the onset of HLA typing, and the phenomenon of LD has resulted in a vast body of evidence that ties certain diseases to a given HLA genotype. However, with ever-improving molecular techniques allowing high-resolution typing, not only are these associations strengthened but they have also allowed fine mapping of HLA loci and/or alleles as being either protective against or increasing susceptibility to a given disease. Identification and targeting of neo-antigens and immunopeptidomes in cancer is the most recent clinical application of HLA, and holds great promise for precision medicine.[31]

Several HLA alleles in South Africans have been identified as associated with protection against, or susceptibility to, a wide variety of diseases which include autoimmune diseases, infectious diseases, and drug-induced hypersensitivity (Table 1). Autoimmune diseases include coeliac disease (CD), rheumatoid arthritis (RA), diabetes mellitus, and various other diseases. Infectious diseases associated with HLA alleles include human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) and tuberculosis (TB). Heterozygosity and certain HLA alleles confer protection in HIV-infected individuals; however, other alleles such as HLA-B*35 have been reported to increase susceptibility to HIV, and are associated with rapid progression to AIDS.[43] A recent study by Ramsuran et al.[44] has shown that increased mRNA expression of HLA-A leads to increased HLA-E expression, which results in increased NKG2A-mediated NK cell inhibition which, in turn impairs targeting of HIV-infected cells, ultimately leading to impaired HIV control. Blocking of HLA-E:NKG2A-mediated inhibition is currently being explored in clinical trials as a possible treatment option for various diseases. In black South Africans, HLA-DRB1*01:02 and HLA-B*58:01 have been associated with hepatotoxicity during HIV combination antiretroviral therapy (cART) initiation in regimens containing nevirapine.[45] This is of particular importance in the context of precision medicine.

In SA, most HLA disease association testing is being done for HLA-B*27. The initiation of molecular typing techniques has shown HLA-B*27:05 to have the strongest association with ankylosing spondylitis.[46] CD has been shown to have a significant association with HLA-DQ2 (DQA1*05/DQB1*02 allele groups) and HLA-DQ8 haplotypes (DQA1*03/DQB1*03:02 alleles). More than 95% of CD patients have HLA-DQ2 and/or HLA-DQ5.[47] The majority of work on HLA disease association has been on autoimmune diseases and especially RA. Scherak et al.[48] reported the association of RA with HLA-Dw4 in 1980. New evidence suggests that RA is associated with RAA shared epitope sequences (positions 72 - 74), modulated by amino acid sequences at positions 70 and 71, resulting in six genotypes with low to high RA risk.[49] The HLA-DR4 as well as the RAA sequence phenomenon has been well described in the black SA populations.[50]

There is a great need to develop and test vaccines that are specific for the diseases that affect southern African populations. The genetic diversity of pathogens together with the genetic diversity of HLA in

Table 1. Summary of HLA-disease associations in South African populations

| | HLA class I | | HLA class II | | |
	Susceptive	Protective	Susceptive	Protective	References
Ankylosing spondylitis	B*27	-	-	-	46, 55
Coeliac disease	-	-	DQ2 DQ5 DQ8	-	47
Graves' disease	-	-	DRB1*01 DRB1*03	-	56, 57
Rheumatoid arthritis	-	-	DRB1*04:01 DRB1*10	DQA1*05:01 DQB1*06 DRB1*03:01 DRB1*03:02 DQA1*01:02	50, 57, 58
Type I diabetes	B*08 B*14	-	DRB1*03 DRB1*04	DQA1*04 DRB1*03:02 DRB1*06:02 DRB1*11	57, 59
HIV	B*08/08:01 B*18/18:01 B*45/45:01 B*51:01 B*58:02	A*74/74:01 B*13:02 B*44:03 B*57:03 B*58:01 B*81:01	-	-	60
Tuberculosis	A*01 B*08 B*27	-	DRB1*03 DRB1*13:02	-	57

HLA = human leukocyte antigen.

these populations are necessary for the development and efficacy of HLA-based vaccines.[51] The majority of HLA frequency studies have focussed on high-income countries, where the prevalence of most infectious diseases is low,[51,52] while countries mostly affected by such diseases would benefit more from these types of studies. HLA frequency studies would need to be extended to lower-to-middle-income countries in order to get an idea of the most common HLA alleles present in populations affected by diseases such as HIV and TB, to name a few. Alleles that occur frequently could be grouped into supertypes and used as targets for vaccines. Supertypes are HLA groups that share peptide-binding specificity. Twelve different supertypes have been identified to date and have been shown to be effective in identifying and characterising T cell epitopes from a variety of different disease targets.[53] Applying the concept of HLA supertypes to vaccines is promising, as supertypes would narrow the search for antigenic peptides that will bind the HLA alleles of a large proportion of the population.[54] This knowledge would be particularly beneficial in populations with high genetic diversity, such as Africans.

Conclusion

South Africa has a high disease burden, including communicable (HIV, TB) and non-communicable (cancer, cardiovascular disease, obesity, diabetes) diseases (http://www.who.int/gho/mortality_burden_disease/en/). There are limited HLA diversity data for South African populations,[19] which affects our understanding of HLA-disease association, donor-recipient matching for transplantation, population genetics and population-specific vaccine design. In the present article, we have reviewed the importance of HLA and its clinical applications for South Africans. Furthermore, we highlight how tools such as HLA imputation might increase our understanding

of HLA diversity in our populations. As access to NGS becomes easier and cheaper, more South Africans may have their HLA genotypes determined; this will better inform disease association studies, donor recruitment strategies in bone marrow registries, and our understanding of human genetic diversity in South Africa in general.

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