What sparked your interest in immunogenetics? Could you outline the objectives of your research?

Immunogenetics is a fascinating field within genetics. The human leucocyte antigen (HLA) region on chromosome six is the most diverse within the genome. This highly gene-dense region is characterised by immense individual gene polymorphism, linkage disequilibrium between the genes and diversity among populations. The highly polymorphic nature relates to its function as an immune response to foreign pathogens, and thus must evolve in a diverse gene system to be able to respond to a variety of immunogenic challenges. However, in transplantation of organs and stem cells, the challenge is to define the best donor to minimise the risk of complications such as rejection, non-engraftment and graft-versus-host disease. To summarise the objectives of our work in three (or five!) words: understanding HLA (and immune-related) polymorphism.

Could you briefly explain the HLA system? How do you study this?

HLA proteins are molecules on the cell membrane of white blood cells (eg. B- and T-cells) that can evoke an immune response. Some lymphoid cells (B-cells) can generate antibodies that react to non-self HLA molecules. Whilst immune responses are generally required to eliminate foreign intruders, following transplantation HLA differences between recipient and donor can also initiate an undesired immune response. Consequently, matching for HLA in stem cell transplantation (SCT) is crucial. Matching grades depend on the HLA allelic diversity, which can be determined by serological approaches (identification of HLA proteins on the cell membrane), molecular approaches (DNA sequencing) and cellular tests to define the functional relevance. Whilst serology and cellular approaches define a limited level of diversity, both are functionally relevant. This is in contrast to DNA gene polymorphism analysis that includes non-functional polymorphic sites. Understanding relevant HLA and immune-related polymorphism is crucial since DNA-based HLA typing technology is the gold standard method used to define full HLA diversity.

How does the sequencing method you currently use differ from conventional Sanger sequencing?

Whilst traditional Sanger sequencing has an important place in current diagnostics, we have developed a methodology to separate the parental chromosomes so haplotype polymorphisms could be defined. This eliminates ambiguous results that are a major problem in HLA typing and commonly associated with conventional heterozygous sequencing approaches. In the 1980s, sequence gels were read as poetry ‘GATC’. In modern systems the sequencing process is integrated and automated, with respect to pipetting, analysis and bioinformatics, to sort and interpret the data. These advanced systems are now firmly established within advanced, contemporary laboratories. Next generation sequencing (NGS) offers extended possibilities for high resolution typing samples of volunteers who are willing to donate stem cells registered in the worldwide stem cell donor databases, assembled in the central database, the Bone Marrow Donors Worldwide (BMDW). Moreover NGS allows for cost-effective sequencing of the entire HLA region.
A HEMATOPOIETIC STEM cell transplant is the transplantation of multipotent stem cells usually derived from the bone marrow or peripheral blood. It is most commonly carried out to treat cancers of the blood or bone marrow. If the procedure is performed early in disease progression it can cure more than 90 per cent of patients. A limitation with its use is the need to identify an immunologically close enough match between patient and donor to avoid the new cells being rejected by the patient’s immune system as foreign invading cells. Since the first successful bone marrow transplant in 1968, extensive research has been conducted worldwide to investigate the interaction between patient-donor immune systems to understand the processes in selecting compatible donors to ensure a positive outcome for the transplant.

The human leukocyte antigen (HLA) is a cluster of genes on chromosome six involved in disease defence and immune system regulation. One of the many functions of their encoded proteins is to present antigens to T-cell receptors, and they are also involved in recognition of ‘self’, to which no immune response is directed. Thus, when identifying an appropriate stem cell donor, it is important to pair a similarly matched HLA phenotype patient and donor so the donated cells are viewed as ‘self’ and not ‘foreign’ invaders that need destroying. HLA genes are among the most polymorphic in the genome; more than 9,500 alleles have been described and characterised. This diversity has evolved to enable a response to the wide variety of immunogenic challenges in the form of foreign pathogens. HLA genes are inherited together; a linkage disequilibrium exists whereby some combinations of alleles are differentially more common in the population. Thus, patients with rare haplotypes – combinations of alleles – may find it difficult to find a donor despite data of the genotype of HLA alleles from more than 15 million potential donors comprising the registry of Bone Marrow Donors Worldwide. Consequently, there is a global need to find and characterise individuals carrying rare HLA haplotypes to improve the success rate of finding a match for stem cell transplant patients.

What challenges have you faced in your research thus far and how have you overcome them?

With the introduction of molecular approaches and more rapid and accurate sequencing techniques, many new HLA alleles and polymorphic sites have been identified. This can be searched for on a database, managed by the ImMunoGeneTics (IMGT) project, which provides allele sequence databases and coordinated nomenclature of the major histocompatibility complex genes. The main question we are faced with is: which are relevant for consideration in matching strategies for SCT? Relevance is determined by immunological responses obtained from serological and cellular data, retrospective analysis of transplanted patients and frequency of the alleles. Understanding and interpreting diversity is a major challenge being addressed in the international HLA and immunogenetics workshops and consortium studies. The next challenge we face is converting this new molecular knowledge, gained from sequencing and analysis studies, into the healthcare environment so it can be used to influence patient care policy.

What are the practical applications of your work? How can your findings be translated into patient care?

The specification of antibodies to particular epitopes refines the reactivity definition of HLA antibodies and can help patients possessing these antibodies to be receptive to the donor in organ transplantation; we have shown that a mismatch at the allele level can still be a match at the epitope level. The effect of individual gene polymorphisms, in relation to functional tests in SCT, is currently being addressed by many research groups. Evidence is emerging that specific amino acids or a combination of amino acids (epitopes) have a more influential role in directing the immune response than the alleles themselves. The impact of this shift in matching criteria should enable prediction of the effect of mismatching and complications after SCT.

Defining permissible donor-patient mismatch in stem cell transplants

A team of researchers studying transplantation immunology at the Maastricht University Medical Center in The Netherlands is considering the relevance and applicability of the new view and extended HLA polymorphism in HLA immunogenetics for finding stem cell donors.
INTELLIGENCE

THE RELEVANCE OF POPULATION HLA IMMUNOGENETICS IN FINDING STEM CELL DONORS FOR STEM CELL TRANSPLANTATION: NEW MATCHING STRATEGIES

OBJECTIVES

To understand the function of human leucocyte antigen (HLA) and immune-related polymorphism in immune responses, disease and transplantation.

KEY COLLABORATORS

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PROFESSOR DR MARCEL TILANUS is head of the Department of Transplantation Immunology and Director of the Tissue Typing Laboratory at the Maastricht University Medical Centre, The Netherlands. He is one of the pioneers who introduced molecular techniques into the HLA typing approaches and developed a routine diagnostic applicability by the sequencing based typing technique; now the gold standard for HLA typing.

THE EVOLUTION OF ALLElic VARIATION IN GAUDELouPE

Guadeloupe is a chain of five inhabited islands in the Caribbean. Throughout history, multiple colonisations, decimations and the introduction of foreign labour forces has caused the population of the islands to evolve great genetic diversity and, with respect to the polymorphic nature of the HLA, there is the potential for a significant number of rare haplotypes to exist within current populations of these islands. The gene pool of the population contains influences of Arawaks from South America, the Amerindian people, Europeans, Indians and Africans. If members of the Guadeloupean population can be screened for rare haplotypes, which can then be characterised and added to the bone marrow donors registry, they could provide a match for patients possessing rare allele combinations who are waiting for transplant but are unable to find a match.

Tilanus’s study involved HLA sequence-based typing of 228 Guadeloupeans and demonstrated that, in line with the historical migration patterns, the allele inheritance patterns had similarities with those from Africa and also demonstrated influence from Indian and Caucasian populations. Through the course of this project novel HLA alleles and haplotypes were identified. The characterisation of these forms the basis of four articles in publication, facilitating discussions concerning the ethnically related allele distribution frequencies. From the results of the Guadeloupean study, Tilanus and his team have been able to conclude that the extent of the HLA allele and haplotype diversity found in the Guadeloupean population merits their inclusion in a bone marrow registry.

A SNP APPROACH TO MATCHING

In addition to increasing the success of matches by including additional rare alleles in the BMDW registry, the Maastricht University researchers also hypothesise that they can improve incidence of tissue matches by identifying single-nucleotide polymorphisms (SNPs) in the HLA region genes. SNPs that represent specific functionally relevant epitopes enable epitope/SNP matching rather than matching at the level of an entire allele. An epitope, many of which are found in any particular allele, is the part of an antigen to which the antibody attaches itself during the immune response. Tilanus explains how this approach can have potential benefits: "The patient/donor can be mismatched at the allele level but matched at the epitope level in the context of the other HLA alleles of the patient, or at its DNA equivalent, SNPs. Different alleles share epitopes and an individual only reacts against foreign epitopes". Thus, focusing the degree of matching specifically on these areas of the gene, which are most heavily involved in any donor-recipient tissue rejection, will allow areas of the gene that are mismatched, but less functionally significant, to be ignored.

To enable this, Tilanus’s laboratory has developed ultra-high resolution sequence-based typing (SBT) to assess all positions of SNPs in the HLA alleles. This approach exploits automated technology to improve efficiency of genotyping and, through carrying out research into the particular HLA loci important for parts of the immune response involved in donor-patient tissue matching, the team is refining the conventional match criteria to focus on functionally significant loci. The traditional sequencing method – Sanger sequencing – is characterised by the frequent occurrence of genotypic and allelic ambiguities in most of the samples tested, which interferes with the accurate identification of particular HLA alleles. The new approach adapts the Sanger sequencing strategy but enables unambiguous typing. It involves the sequencing of whole genes on separated chromosomes rather than just a number of exons that are most significant, using the results of low resolution typing as reference to separate the parental chromosomes. This strategy has now proven its value and is used as the routine HLA typing strategy within the clinical setting, enabled by its easy, cost-effective and user-friendly implementation in any diagnostic HLA lab that has the existing potential to conduct Sanger sequencing.

The success of this project would not have been possible without the multidisciplinary approach arising through collaborations with leaders in population genetics, transplantation specialists, bioinformaticians, clinicians and health psychologists. Progress in Guadeloupe – resulting in the addition of rare allele donors to the BMDW – has led to the application of this study to similarly genetically diverse populations in distinct Taiwanese tribes and Estonia due to the context of its independence and history of occupations.