Narinder Mehra Interview

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Professor Narinder Mehra is a researcher at the All India Institute of Medical Sciences. His research interest lies in all aspects of the human MHC, ranging from its potential role in defining biomarkers of disease associated genes, evaluating genetic diversity of HLA and other immune associated genes. Prof. Mehra recently published a free eBook on the clinical relevance of antibodies in solid organ transplantation. The book has 16 chapters that provide state of the art information under the following themes: (*i)* HLA sensitisation and matching strategies to improve clinical outcome (*ii*) technical, functional and clinical impact of HLA and non-HLA antibodies (*iii*) cellular aspects of antibody responses and others.

Prof. Mehra recently spoke to the Immunopaedia team about the immunology behind transplantation and how his research has contributed to the field.

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Why is understanding of Immunology important in organ transplantation? Transplantation is the treatment of choice for functional failure of many organs. The success of an organ graft depends on the degree of histocompatibility between donor and recipient, amongst other factors. This genetic difference in man is determined by a set of immune response genes clustered together as human leukocyte antigen (HLA) system. These play an important role in immune discrimination between self

and non-self (foreign) molecules.

Although efforts are made to ensure that the patient and donor are matched for HLA gene loci as completely as possible, this is difficult to achieve in most instances. Hence immunosuppressive drugs are given to control the host immunity caused by HLA disparity. The aim is to prevent or control rejection and allow the recipient to develop long-term acceptance of the graft.

Major factor responsible for graft rejection is T-cell recognition of the genetically encoded donor alloantigens by the immune system. The CD4+ recipient T-cells recognize donor allo-antigens through either the 'direct pathway' in which host T-lymphocytes recognize native MHC molecules expressed on graft-associated APCs or the 'indirect pathway' in which the donor alloantigen-derived peptides are recognized in the context of self-MHC molecules expressed on recipient APCs. The direct pathway is of particular relevance during acute rejection. Donor derived APCs expressing donor alloantigens rapidly migrate from the graft and enter the secondary lymphoid tissues, where these can encounter and prime the allospecific T-cells. Cytotoxic T-lymphocytes, B-cells, macrophages and natural killer cells are recruited in a graft undergoing rejection. The main mechanisms implicated for graft destruction are cell-mediated cytotoxicity, delayed type hypersensitivity and antibody-dependent cellular cytotoxicity.

Rejection can be mediated by antibodies, lymphocytes or both and can manifest as hyperacute (in the early post-transplant period, within hours), acute (occur at any time) and chronic rejection (slowly developing process with progressive decline in graft function).

Hyperacute rejection is caused by preformed donor- specific antibodies (DSA) in a pre-sensitized recipient. Humoral pre-sensitization may be caused by a previous transplant, blood transfusions or pregnancy. The alloantibody-mediated rejection is initiated by activation of the complement cascade leading to release of various inflammatory mediators and the initiation of the coagulation and fibrinolytic systems. Hyperacute rejection is manifested by rapid vascular constriction, oedema and thrombotic occlusion.

What factors determine whether someone will accept or reject an organ? Development of donor specific anti HLA antibodies, either preformed or *de novo* occurrence following solid organ transplantation is of prime importance leading to graft rejection. The spectrum of antibody mediated insult ranges from hyperacute rejection to chronic antibody mediated rejection (AMR) leading ultimately to graft loss. A meticulous 'cross-match test' for possible presence of anti-donor antibodies is always performed before any transplantation. This is similar to the direct cross-match done prior to blood transfusions; donor lymphocytes instead of red cells are tested with patient serum. Several improved cross-match assays have been developed to increase the sensitivity and specificity of the test. These include the antihuman globulin test (AHG), flow cytometry and solid phase assays like enzyme-linked immunosorbent assay and Luminex systems. Serum treatment with dithiothreitol is used to distinguish clinically less relevant immunoglobulin M (IgM) type antibodies.

The discovery of solid phase technologies, particularly the single antigen bead (SAB) assay which allows detection and characterization of HLA antibodies with high degree of sensitivity and specificity has highlighted the relative importance of antibody mediated rejection. Recent evidence suggest that AMR is the major player responsible for graft loss, causing more than 60% of late kidney graft.

While anti-HLA DSAs have been widely associated with poor graft survival, the role of non-HLA antibodies, particularly those directed against endothelial cells, is beginning to be realized. Of these, MICA (Major Histocompatibility Complex class I chain related molecule A) antibodies are the most notable because of their potential in promoting hyper acute and/ or exaggerated antibody mediated rejection.

While the role of acquired immunity in graft rejection is well established, the potential of innate immunity playing an important role in graft outcome is gaining importance. MICA is a potent activator of NK cells via their receptor NKG2D leading to activation of the NK cell mediated arm of allograft rejection.

Human leukocyte antigen antibodies that develop *de novo* post-transplantation and may not necessarily be donor- specific are also associated with poor graft survival. It is, therefore, advisable to monitor routinely the post- transplant development of such antibodies, as a predictive marker for allograft function. Recent advances in immunological techniques promise improved management of transplant patients by predicting rejection episodes before the onset of irreversible and terminal damage.

HLA matching is most beneficial for both short- as well as long-term survival of the renal allograft using live related or deceased donors. The poor match status between donor and recipient is frequently associated with more vigorous antibody response. Molecular approaches for clinical monitoring of the post-transplant immune status and identifying the best functionally matched donor before the transplant procedures are among the key areas of futuristic research in the area of clinical transplantation. Continuing progress in understanding molecular mechanisms of graft rejection may lead to the ultimate goal of long-term acceptance of organ allograft through tolerance induction.

What is HLA and what role does it play in organ transplantation? The histocompatibility antigens are cell surface glycoproteins expressed on nucleated cells whose major function is to bind peptides within the cell or from outside and present them at the cell surface for inspection by T cells of the immune system. These antigens are a part of the Major Histocompatibility complex (MHC), which exists in most vertebrate species and whose products were originally defined as the most crucial elements controlling graft rejection. Human MHC research has revealed HLA to be the most polymorphic genetic system in the mammalian genome, and this unique diversity parallels the need for diverse peptide sites within the MHC to combat a fast-evolving range of pathogens. Clinical screening of HLA polymorphism is now an important prophylactic biomarker system for various diseases and a useful tool for development of peptide-based vaccination approaches.

Besides their major role in donor selection for organ and bone marrow transplantation, other important areas in which HLA has provided great help include paternity determination, identification of susceptibility or predisposing genes for a wide variety of diseases, particularly those with infectious and autoimmune etiology, prediction of 'risk' development for disease in families, anthropological characterization of different races and ethnic groups and for understanding the control and regulation of the immune system.

The major biological function of the MHC is to bind peptides for presentation to T cells. Following discovery in the early 1970s by Benacerraf and Hugh McDevitt of the immune response genes and their mapping within the class II region of the mouse H-2 system, the cellular recognition of foreign antigens was explained through elegant experiments performed by Rolf Zinkernagel and Peter Doherty. Their studies revealed that cytotoxic T cells could recognize a virus-infected target only in the context of class I molecules, a phenomenon called 'MHC restriction'. For their pioneering discovery about the T-cell antigen recognition system, and the biological function of the MHC, Zinkernagel and Doherty received the Nobel Prize in Medicine and Physiology for the year 1996.

Human MHC is the major barrier for selection of an optimally matched donor for patients requiring solid organ transplantation. The probability of finding a 100% matched donor is higher among family members, because of the haplotypic inheritance pattern of the MHC. Matching strategies for solid organ transplantation consider only HLA-A, -B and -DR loci. In renal and other solid organ transplantation, a direct relationship exists between graft survival and the level of HLA matching.

What role could our understanding of Genomics play in Transplantation? Transplantation medicine has saved numerous lives after the first successful solid organ transplantation in 1954 performed by Joseph Murray and his team at the Brigham Hospital at Boston, United States. Since then there has been no looking back. However, two main challenges are still faced by transplant scientists worldwide; the first being the low rate of long term graft survival which has remained unchanged for the last 30-40 years and second being the mortality rates of allograft recipients, which are still significantly higher than normal population. The new domain of research based on OMICS studies also known by the name of 'Transplantomics' focuses to address these challenges.

In attempts to prevent/delay allograft rejection/loss, therapeutics are primarily focused on manipulations of the recipient's immune system by blocking known signaling pathways or otherwise inactivating immunologic processes. Hence, currently the immunosuppressant regimen is customized for the patient and dosage is titrated up or down to achieve effect and minimize toxicity. This decision making process is based on the clinical judgment of the physician. Dose adjustments are targeted towards achieving "ideal" concentration of the drug within the bloodstream, on the supposition that ideal drug concentration will correspond with ideal drug effect. The current practices in post-transplant monitoring involve the invasive biopsies and that too when the damage to the allograft is almost irreversible. Attempts are currently underway to identify markers that can predict graft prognosis thus providing a window of opportunity for necessary intervention before the graft turns redundant. These attempts have been reinforced by recent discoveries in the field of molecular medicine which include areas like genomics, proteomics, etc. and have widened the horizons to

identify newer markers to understand the pathophysiology. The bulk of investigations in omics in transplantation have been based on microarray studies of the transcriptome.

Omics tools can be used either for biomarker discovery or for elucidation of the molecular mechanisms underlying pathophysiologic processes. Peripheral blood contains almost exclusively recipient-derived cells, and genetic profiles are recipient-driven. Transcriptomics, proteomics and metabolomics research in peripheral blood is promising for both biomarker and therapeutic target discovery. Genotype analysis in kidney biopsy samples gives information on the donor genotype, although extensive graft inflammation with recipient-derived cells could also lead to the presence of recipient's DNA in biopsy samples. Omics analyses in biopsy specimens can be used for both therapeutic target studies and for biomarker studies, although the invasive procedure represents a major drawback of potential tissue-derived biomarkers. Finally, urine analysis using omics tools offers a great window for biomarker discovery.

Identification of relevant biomarkers help in understanding of the underlying pathological changes taking place at the molecular level enabling better prediction of the individual's allograft status and can facilitate personalized transplantation medicine, leading to long-term graft survival. The discovery of biomarkers in solid organ transplantation integrate information from multiple platforms such as genotype analyses of singlenucleotide polymorphisms (SNPs), epigenetic studies and analyses of mRNA, microRNA (miRNA). These also include protein (proteomics) peptide (peptidomics) antibody (antibodyomics) and metabolite (metabolomics) profiling. Highthroughput analyses are becoming more accessible, affordable and customizable, and rapid developments in analytical tools now allow integrated metaanalyses of different datasets across different experiments, platforms and technologies.

How has your research contributed to the field of clinical immunogenetics and transplant immunology? I have been involved in research and development of almost all aspects of the human MHC ranging from its potential role in defining biomarkers of disease associated genes, evaluating genetic diversity of HLA and other immune associated genes (chemokine, cytokine gene polymorphism, killer inhibitory immunoglobulin receptors and others) at the population level and immunological aspects of organ and hematopoietic stem cell transplantation. We made original contributions on the immunogenetic aspects of mycobacterial, autoimmune and rheumatological diseases leading to a better understanding of their molecular basis of susceptibility. Currently, my focus is on the study of immune mediators in organ and hematopoietic stem cell transplantation, genome diversity of HLA at the population level and towards developing MHC based vaccination approaches in tuberculosis and other mycobacterial infections. We launched a major program in Molecular Medicine and HIV research with focus on studying genetic polymorphism on a host of immunomodulatory genes. Besides the above, I established the 'Indian Marrow Donor Registry (IMDR)' which has proved most valuable in the area of hematopoietic stem cell transplantation.

I started my scientific career in the mid 70's under the guidance and in collaboration with Prof Jon vanRood of Leiden, Holland. My first and internationally well known breakthrough was in the field of **mycobacterial diseases**, when we showed for the first time that the HLA-DR2 linked genes control

the type of immune response that develops following *M. Leprae* infection and thus guide the development of pauci versus multibacillary disease. We were indeed the first to conduct multi case family studies that conclusively established an HLA linked control of immune response in leprosy.

Later, my group in New Delhi defined the peptide binding motif in the MHC through sequence based analysis. Our studies revealed that presence of Arginine rich motif in 'pocket 4' and valine/glycine dimorphism V^{86} in 'pocket 1' of the peptide binding groove of the MHC plays an important role in the overall disease expression and immune responsiveness following *M. Leprae* and M. tuberculosis infection. Besides MHC, we also defined the role of innate immunity markers, chiefly the Toll like receptors (TLRs). His studies provided evidence to suggest that specific MHC phenotypes play a critical role in the development of infection in drug resistance forms of pulmonary tuberculosis by inducing anergy/unresponsiveness to *M.tuberculosis* leading to spurt in bacillary population similar to the situation in lepromatous leprosy.

The work on Immunogenetics of infectious diseases led us to defining Ir genes that control HIV transmission, resistance and disease progression. Through a multicentric approach, observations on the MHC genome, chemokines and their ligands as well as cytokine gene polymorphism analysis suggested a possible fast progression of HIV infection to AIDS in the Indian population.

In addition to the infectious diseases, we performed in depth analysis of the genetics of Type 1 Diabetes and other autoimmune diseases. Utilizing a genomic analysis approach involving a number of microsatellites and SNPs, we reported that rather than a single HLA-DR3 positive haplotype, the Indian population is characterized by multiplicity of such disease associated autoimmunity favouring genes, each of which are positively associated with type 1 diabetes and celiac disease.

In the area of Transplant Immunology, we investigated the immunogenetic factors, cellular basis and predictors of acute graft – versus – host disease in bone marrow transplantation as well as the antibody basis of graft rejection following renal transplantation. We established robust technologies and quality controls for defining anti donor, anti HLA as well as anti MICA antibodies in solid organ transplantation. Our more recent investigations have suggested that a comprehensive screening and meticulous evaluation of the sensitization status of renal allograft recipients both in the pre as well as post transplant periods is of critical value for optimizing long term survival of the graft. Based on our contributions, the Frontiers in Immunology invited me to edit their important Research topic highlighting the clinical and biological significance of antibodies in solid organ transplantation.

For me personally, it has been an academically satisfying journey through the world of HLA and to be known internationally as a prototype from India in the area of Transplant Immunology and Immunogenetics. It is due to my love and passion for the subject and the honesty of purpose that I could receive several of the high awards for Science in India. These include the coveted S.S. Bhatnagar prize of the Council of Scientific and Industrial Research (CSIR), Tata Innovation Fellowship of the Department of Biotechnology (DBT) and the Dr B.R. Ambedkar award of the Indian Council of Medical Research (ICMR). I have also been honoured internationally i) as the 'Chevalier of the National Order of Merit' by the President of France, ii) the President of Iran selected me for their highest

science award, the Khwarizmi Proze, iii) the Hungarian Academy of Sciences elected me as their 'foreign fellow' from India, iv) I was four times elected as the Councillor for the International Union of immunological societies (IUIS), v) Founder Secretary General of the Federation of immunological societies of Asia-Oceania (FIMSA), vi) The World Academy of Sciences (TWAS) elected me as their fellow and this is considered to be an important academic award, among many others.

I have been a member or chairperson of numerous scientific committees and boards internationally. These have been very challenging roles as I had to share the dias with the highly acclaimed international scientists including the Nobel laureates and prove myself to be worthy of the same. I get the most satisfaction and happiness when my peers comment that I have been able to successfully cross many hurdles and challenges, while working under difficult conditions due to lack of proper funding and mentoring. For me, crossing one challenge always creates several new opportunities. These are two sides of the same coin. I have tried to be true to my self and to the science that I have done.

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