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Julio C. Delgado and Eszter Lázár-Molnár

Human Leukocyte Antigen Typing by Next-Generation Sequencing 565

Tracie Profaizer and Attila Kumánovics

Human leukocyte antigen (HLA) allele ambiguities are the result of limitations of current HLA typing methodologies. Ambiguities maybe due to polymorphisms in unsequenced regions of HLA genes or cis/trans variants that cannot be distinguished by Sanger sequencing. Next generation sequencing (NGS) can resolve these two sources of ambiguity because the entire gene can be sequenced. Commercially available HLA NGS genotyping kits enable laboratories to deliver high-quality and unambiguous HLA typing results at an affordable cost. Third generation sequencing technologies are poised to further improve sequencing quality, shorten turn-around and library preparation times, as well as provide full-gene phasing.

Technical Aspects of Crossmatching in Transplantation 579

Andrés Jaramillo, Daniel S. Ramon, and Scott T. Stoll

The presence of antibodies directed against HLA molecules expressed on the donor's cells is one the most important risk factor for serious clinical complications after transplantation. The lymphocyte crossmatch is one of the most important tests available to the laboratory as this assay detects the presence of donor-specific anti-HLA antibodies in potential allograft recipients. Early crossmatch methods used a complement-dependent cytotoxicity test, which was useful for detecting anti-HLA antibodies responsible for hyperacute graft rejection but lacked adequate sensitivity and specificity. Consequently, more sensitive and specific crossmatch methods were developed ultimately leading to the flow cytometry crossmatch as the preferred methodology.

Human Leukocyte Antigen Epitope Matching in Solid Organ Transplantation 595

Matthew F. Cusick and Peter T. Jindra

HLA epitope matching provides a better approach to stratify patients at risk of developing antibody-mediated rejection compared with counting HLA mismatches. However, several immunologic parameters are not incorporated into these algorithms used to assess HLA epitopes, raising questions about the predictive value of these programs. Therefore, it is imperative to obtain more 3D structural data of antibody-antigen binding to "train" these computer algorithms. Also, mechanistic studies should be performed to prove these theoretic "epitopes." Most important, more information is needed to ensure these predictive computer algorithms are equitable and safe to use in clinical diagnostics before wide-scale implementation.

Maintaining the Health of the Renal Allograft: Laboratory and Histologic Monitoring After Kidney Transplantation 607

Carrie A. Schinstock and Manish J. Gandhi

Advances in posttransplant care, including new immunosuppressive medications have led to excellent short-term renal allograft survival. However, there is a small therapeutic window within which the patient and the clinician must balance the risk of rejection, with side effects such as infection, malignancy, and toxicity. Laboratory testing plays a key role in this ongoing monitoring, which includes relatively simple tests, such as serum creatinine, to complex tests, such as solid-phase assays, used to monitor for donor-specific antibody and surveillance allograft biopsies. This article reviews the role of the laboratory tests and surveillance biopsies in posttransplant monitoring.

Recent Advancements in the Assessment of Renal Transplant Dysfunction with an Emphasis on Microarray Molecular Diagnostics 623

Meagan Barner, Jenefer DeKoning, Zahra Kashi, and Phillip Halloran

Conventional assessment of renal transplant rejection and injury through use of histology, C4d staining, and HLA antibody testing, has been the standard approach to transplant management. By many measures, these methods of conventional assessment may be considered flawed, particularly with the subjective nature of histologic diagnoses. The Alberta Transplant Applied Genomics Center has developed the Molecular Microscope diagnostic system, which uses microarrays to measure gene expression. These data are analyzed using classifiers (weighted equations) that compare the tested biopsy to a proprietary reference set of biopsies to provide objective measures of the status of the renal transplant.

Diversity of Killer Cell Immunoglobulin-Like Receptors and Disease 637

Raja Rajalingam

Natural killer (NK) cells use variable inhibitory and activating killer cell immunoglobulin-like receptors (KIR) to detect and eliminate virally-infected or tumor-transformed cells. The effector function of a given NK cell depends upon its KIR receptor-repertoire, HLA ligands used for licensing at the time of its development, and ligands it senses on the targets. Genes encoding KIRs and HLA ligands are located on different chromosomes, and feature substantial variations. Independent segregation of these genes results in variable KIR-HLA inheritance that would contribute to the individual's immunity. This review describes KIR-HLA diversity and summarizes current knowledge on their implication in disease associations.

The Role of Human Leukocyte Antigen in Celiac Disease Diagnostics 655

Eszter Lázár-Molnár and Melissa Snyder

Celiac disease is an autoimmune disease affecting the small intestine, triggered by gluten sensitization in genetically susceptible individuals worldwide. Celiac disease development is strongly linked to the presence of HLA-DQ2 and/or DQ8, which present the immunogenic gluten peptides and trigger the immune response leading to pathogenesis. Because of

the variability of clinical symptoms, the disease is often underdiagnosed. Intestinal biopsy and the presence of antibodies to deamidated gliadin and tissue transglutaminase are recommended diagnostic tools. Genetic testing for HLA DQ2 and DQ8 can be used to rule out disease in at-risk populations.

Human Leukocyte Antigen Associations in Drug Hypersensitivity Reactions **669**

Ryan J. Schutte, Yonghu Sun, Danmeng Li, Furen Zhang, and David A. Ostrov

Severe adverse drug reactions are a common cause of morbidity and mortality. Some of the most severe reactions are immunologically mediated and have been linked to specific HLA alleles. The mechanisms underlying HLA-associated drug hypersensitivity are complex and not fully understood. Recent findings have provided insight into recognition mechanisms underlying drug-induced immunopathogenesis and criteria for increasing positive prediction of hypersensitivity. Refining pharmocogenetic testing strategies to better identify at-risk individuals can improve hypersensitivity prevention and mechanism characterization.

Human Leukocyte Antigen and Disease Associations: A Broader Perspective **679**

Mengkai Shieh, Nilesh Chitnis, and Dimitri Monos

HLA molecules play a significant role in immunity and disease susceptibility. GWAS studies underline the critical role of the MHC region in a wide range of diseases and remind us that the HLA genes, included within the MHC, interact extensively with other genomic regions which influence their functions. Recently, MHC/HLA genomic sequences encoding for miRNAs have been reported to interact with targets within and outside the MHC, influencing the expression of many transcripts. High throughput sequencing technologies provide unique opportunities for complete HLA/MHC sequence characterization, helping to elucidate their interactive relationships in a plethora of physiological and disease processes.