MEETING REPORT

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ESH Euroconference on Translational Research in Transplantation: immunogenetics, pharmacogenomics, proteomics, and immunobiology

July 4–6, 2006, Paris, France

The ESH Euroconference on Translational Research in Transplantation was held July 4–6, 2006, in Paris, France. It was organized by the European School of Hematology to discuss the state of the art and perspectives of transplantation research. The chairpersons of the conference were Dominique Charron (INSERM U396, Institut Universitaire d'Hematologie, Paris, France) and Jon J. van Rood (Europdonor Foundation, Leiden, the Netherlands). Major discussion topics were the future directions for basic science and clinical research.

Our understanding of the diversity of the HLA system is currently undergoing a gradual evolution from practical issues regarding optimal donor choice to the investigation of the properties of individual HLA molecules in the context of antigen presentation and tolerance induction. After the sequencing of the human genome, it has become apparent that MHC genes represent the most polymorphic system in humans. At the same time, evidence of associations of HLA molecules with the occurrence of numerous diseases (mainly autoimmune diseases) is still growing. Therefore, as discussed by John Trowsdale, research should concentrate on the mechanisms of how certain HLA genes (possibly determined by modern methods, such as sequencing and single-nucleotide polymorphism analysis) affect pathological conditions. A number of gene clusters have been found to be associated with the MHC gene complex encoding, for example, tRNA, histones, zincfinger transcription factors, and olfactory receptors. Therefore, paired with certain HLA molecules, other different polymorphic genes which may influence human physiology are being inherited.

The investigation of HLA functions has led to a growing interest in NK cell killer inhibitory receptors (KIRs), which recognize certain HLA molecules. KIR genes are also polymorphic, and KIR typing, especially in hematopoietic stem cell transplantation (HSCT), is currently performed in many laboratories. However, there is still no consensus on their importance in transplantation, and the interpretation of typing results is still an unsolved problem. Dr. Trowsdale suggested that future studies should be directed towards finding associations between pairs of HLA alleles and KIR gene alleles (encoded on distinct chromosomes) and clinical observations of transplanted individuals.

Some of the major presentations discussed the role of non-classical HLA molecules in transplantation. HLA-E molecules may be encoded by two alleles: HLA-E*101 (recessive) and HLA-E*103 (dominant). The pattern of HLA-E expression can be recognized by NK cell receptors and influence their activation. This may lead to the significantly higher number of miscarriages in women with the HLA-E*101 allele. However, HLA-E molecules, as shown recently, may present some peptides, such as MHC signal sequences, to T lymphocytes. The observation that the HLA-E*103 allele may protect some people against HIV infection led to investigation of the role of HLA-E polymorphism in unrelated HSCT outcomes. Ryad Tamouza provided evidence that the donor HLA-E*101/ HLA-E*101 genotype has significant impact on the occurrence of severe bacterial infections and early transplant-related mortality. However, similar studies of cord blood transplantation did not confirm these observations.

Hereditary hemochromatosis protein molecules (HFE) molecules, which belong to a group of non-classical MHC molecules, are known for its impact on iron metabolism. Although HFE does not present antigens, it may be directly recognized by allogeneic T lymphocytes. Therefore HFEs, as other polymorphic non-classical MHC molecules, may behave as major histocompatibility antigens, independently of their antigen-presenting properties. As suggested by Francois Lemonnier, the process of allo-recognition of classical MHC molecules may also be independent of the alloantigens presented by them and result in the stimulation of multiple alloreactive T lymphocytes during the acute phase of transplant rejection.

Ernst Holler reviewed current concepts on the impact of immunomodulatory gene polymorphisms on the occurrence of graft-versus-host disease (GvHD). The violent immune system reaction to a conditioning regimen (high-dose chemo- and radiotherapy), also known as the "cytokine storm", does not only have an influence on the antigen-presenting properties of both

the dendritic cells (DCs) and epithelial cells of the graft recipient, but also constitutes a mechanism of immune--cell attack during the effector phase of GvHD. People differ in the amounts of cytokines secreted in response to the trigger, which is explained by cytokine gene polymorphisms among individuals. The influences of IL-10, IFN-γ, IL-6, and also vitamin D receptor and estrogen receptor polymorphisms on GvHD have been already revealed.

Ernst Holler noted the correlation between the injury to epithelial surfaces caused by the conditioning regimen (enabling microbial invasion) and immune attack to the epithelium during GvHD. This may be caused by stimulation of immune system cells by, for example, gut flora via so-called pattern-recognition receptors (PRRs). PRRs, such as Toll-like receptors (TLRs) and intracellular nucleotide digomenzation domain/caspase recruitment domain (NOD/CARD) receptors, are used by immune-system cells to nonspecifically recognize characteristic evolutionarilly conserved motifs of microbial pathogen structure. Therefore, polymorphism of PRR may significantly influence GvHD. It has been shown that TLR4 mutations may reduce the occurrence of acute GvHD, but increase the incidence of Gram-negative bacteremia. Depending on the donor or recipient source of a mutated NOD2/CARD15 gene, divergent results in GvHD have been obtained. Dr. Holler suggested that in the future there will be a possibility to assess the "genetic risk factors" in the donor and recipient prior to HSCT to avoid GvHD and to sustain the graft-versus-leukemia (GvL) reaction.

It was suggested that future analyses prior to transplantation may need specialized software to cope with the growing list of factors influencing transplantation outcome (both clinical and molecular). A prototype of such a program is "HLA-matchmaker". It may be used for optimal donor-recipient selection to minimize the risk of antibody formation against allogeneic HLA molecules. It analyzes the sequences of HLA molecules, picking out fragments that may induce alloantibody response formed by series of amino-acid triplets serving as separate epitopes. Then it compares different HLA molecules according to the incidence of certain triplets. Some mismatched HLA molecules may not possess triplets the transplant donor and recipient do not have in common and therefore may not induce alloantibody formation.

A series of lectures addressed the issue of regulatory T cells (T_{reg}) and their role in immune tolerance and the GvHD or GvL effect. There is still a consensus that most of them may be characterized by the $CD4^+CD25^{high}$ phenotype, but the resulting population is very heterogeneous. However, the finding that FOXP3 protein is a common feature of T lymphocytes possessing immunoregulatory properties is a breakthrough in research of T_{reg} biology. Interestingly, T_{reg}, as presented by Nicole Suciu-Foca, may transfer their tolerogenic properties to other lymphocytes, influencing the phenotype of antigen-presenting cells (APCs). T_{reg} ($CD8^+CD28^-FOXP3^+$) interact with APCs, causing them to express ILT3 and ILT4 factors (immunoglobulin-like transcripts) that render the contacting alloreactive $CD4^+CD45RO^+CD25^+$ T lymphocytes tolerant. Also, the future application of recombinant ILT factors in the clinic to induce transplant tolerance was suggested. Clinical studies revealed that T_{reg} ($CD8^+CD28^-FOXP3^+$) cannot be found in the peripheral blood of patients who reject transplanted organs. Therefore, the group of patients with high numbers of T_{reg} could possibly benefit from a reduction in immunosuppression. In the case of HSCT, as discussed by Matthias Edinger, T_{reg} of donor origin do not contribute to GvHD, but even counteract this reaction. What is of special importance is that T_{reg} do not cause generalized immunosuppression, maintaining an important GvL effect of donor T lymphocytes. Therefore, adoptive transfer of donor T_{reg} may be a future strategy in preventing GvHD after HSCT.

T_{reg}, like no other population of activated T lymphocytes, do not produce IL-2, but can be characterized by constitutive high expression of IL-2Rα (also known as CD25 antigen). Therefore, IL-2, which was considered earlier to have strong immunostimulatory properties (e.g. it is used for tumor immunotherapy), potently activates immunoregulatory mechanisms and influences immune tolerance. Acting directly on T_{reg}, IL-2 stimulates their proliferation and enhances their suppressive properties. Moreover, absorption of IL-2 (usually present at low concentration) by the high-affinity receptors IL-2Rα on T_{reg} may lead to termination of the immune response.

One of the most exciting findings of recent years regarding antigen presentation is the discovery of a novel so-called "semi-direct" pathway of alloantigen presentation. The direct pathway involves the stimulation of the recipient's lymphocytes by APCs of donor origin. This is especially efficient because of the abundance of costimulatory molecules on the donor's APCs, but terminates quickly as these cells become depleted. The indirect pathway involves the presentation of alloantigens (mainly epitopes of the donor's MHC molecules) by the recipient's APCs. The indirect pathway seems to be essential for transplantation failure and therefore, as suggested by Nuala Mooney, should be the main target of new treatment modalities. However, based on these observations, different populations of the recipient's lymphocytes would be activated via direct and indirect pathways: recognizing allogeneic MHC molecules or alloantigens presented by autologous MHC molecules. It was reported recently that the recipient's DCs move within the transplanted organ, acquiring at their surface MHC class I molecules coming from the donor's DCs or epithelial cells. Such naturally modified DCs may stimulate both $CD4^+$ T lymphocytes with direct specificity and $CD8^+$ T lymphocytes characterized by indirect specificity. This is called the "semi--direct" pathway of antigen presentation.

The close interaction between APCs and T lymphocytes, known as the "immune synapse", seems to be the target of future immunosuppressive therapy, as introduced by Christopher Rudd. Agents which could selectively influence this interaction would have strong impact on allogeneic response in the course of solid organ or HSCT. Recent investigations of the CTLA-4 molecule (which inhibits T cell activation) indicated that it decreases the stability of immune synapses. Therefore, the higher expression of CTLA-4, the shorter the time of interaction between DCs and potentially alloreactive T lymphocytes, which decreases the possibility of cell activation. Dr. Rudd suggested that utilizing CTLA-4 molecules in therapeutic strategies against, for example, GvHD or autoimmune disorders should be a subject to consider.

Antoine Toubert discussed the importance of thymic function assessment prior to HSCT. The methodical approach is based on the measurement of so-called T cell receptor excision circle (TREC) concentration in the peripheral blood lymphocytes of the transplant recipient. TRECs are formed in the process of TCRα locus rearrangement due to deletion of the TCRδ region. Proper thymic function seems to be essential for correct immune system reconstitution after HSCT. A high level of TRECs in HSCT recipients seems to be associated with better survival, decreased occurrence of GvHD, and also diminished frequency of bacterial and cytomegalovirus infections. Therefore, the evaluation of thymic function may be helpful in anticipating transplant outcome and also in the follow-up of patients, who may require strict monitoring of their immune system parameters.

Proteomics may, in turn, help in the early diagnosis, progression assessment, and even the prediction of acute GvHD. Eva Weissinger commented on a new method of capillary electrophoresis-mass spectrometry (CE-MS) which is used to detect the pattern of proteins in a patient's urine samples. This pattern seems to be characteristic for different disorders. The multicenter study revealed that using CE-MS it is possible to detect GvHD even up to 14 days prior to the appearance of clinical symptoms.

The lecture held by Willem Fibbe, focused on the issues of mesenchymal stem cells (MSCs), also contained a message that can be interpreted as a warning against reckless and irresponsible application of novel basic research findings in clinical practice. MSC transplantation is currently considered to be an option in future transplantology and regenerative medicine because of MSCs' potential to differentiate into diverse tissues and their immunomodulatory properties. The application of MSCs may counteract GvHD, acting suppressively on the donor's T lymphocytes, which has been evaluated in recent clinical studies. However, Dr. Fibbe showed in a murine model that MSCs which had undergone several divisions *in vitro* formed malignant sarcomas when injected into the tail vein. Subsequent cytogenetic analysis of these cells revealed multiple chromosomal abnormalities, confirming malignant transformation of these cells. It is not difficult to envision an analogous situation in the clinic. This confirms the need for special precautions and the development of assessment protocols when introducing biological products into the clinic.

Current research into transplantology is involved not only in careful analysis of risk factors and appropriate donor choice, but also in the development of modern immunomodulatory therapies and early prevention of adverse events and graft rejection. The conference confirmed the need for a multidisciplinary approach to transplantation, exploiting observations from basic science, especially in the fields of immunogenetics, pharmacogenomics, proteomics, and immunobiology.

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