# Mediating an Immunological Truce

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**Transplants can save lives, but only if the donor tissue is compatible with the recipient's body. Understanding how the body identifies and rejects foreign tissue is crucial for success. The molecules responsible for this are known as major histocompatibility complex (MHC) proteins**

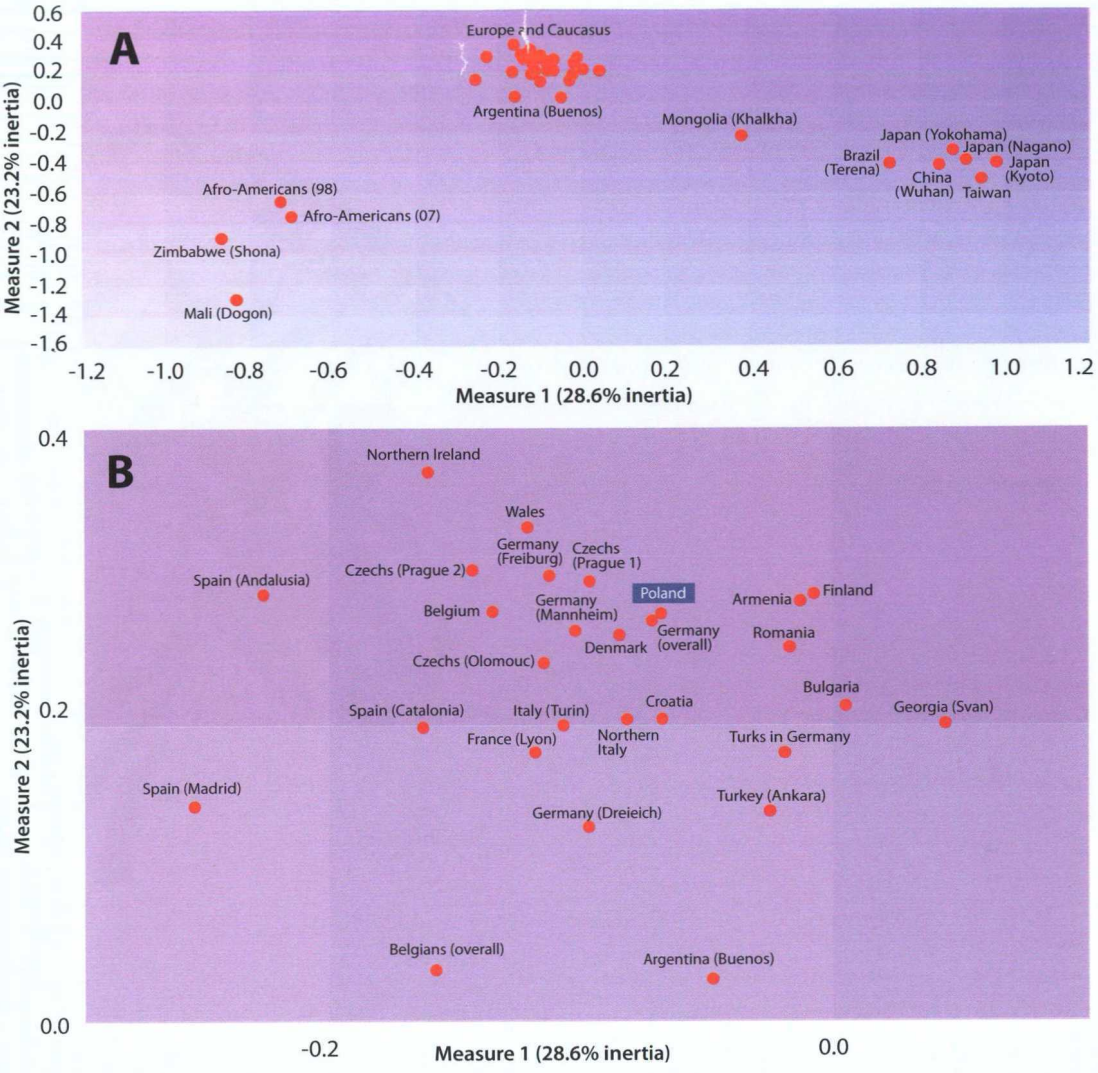
MHC proteins are cell surface molecules. Their task is to present antigens, which are small products of protein degradation inside the cell, to specialized cells of the immune system. MHC proteins were discovered in studies on transplant rejection in mice, conducted by G. Snell, B. Benacerraf and J. Dausset in the first half of the 20th century. In 1980, they were awarded the Nobel Prize for their work. Later, it turned out that MHC molecules are also involved in the immune response against viruses, and R. Zinkernagel and P. Doherty won the Nobel Prize (1996) for discovering that the immune response is limited to all body cells which display MHC molecules on their surface, neglecting foreign MHC-incompatible or self MHC-deprived cells. This is called immunological restriction.

MHC molecules on body cells work closely with a type of immune cells called T cells. T cells can recognize antigens presented by MHC. Antigens can derive both from proteins normally found in the body cell or from intracellular pathogens, such as viruses, which attack this cell. Once the antigen is recognized as foreign, the T cell lymphocyte initiates an immune response against the cell presenting it. MHC molecules, in humans also called HLA (human leukocyte antigen) molecules, are therefore a significant component of the "immunological self," the system distinguishing the body's own structures from foreign structures.

The repertoire of HLA molecules is broad. There are thousands of alleles (variants of genes encoding HLA molecules) in the human population, and each ethnic group has its own characteristic allele combination and frequency. Our team has performed studies to determine the genetic similarity between unrelated individuals listed in the bone marrow donor registry (the closer donor-recipient HLA match, the bigger the chance that the transplant will not be rejected and that graft versus host reaction will not occur). We have shown that the genetic distance determined on the basis of HLA-A, -B, and -DRB1 allele frequency distributions reflects the geographic distance between populations, and a map created on the basis of the gene frequency distribution closely resembles a geographic map. The results are shown in the figure on the next page - the reader can check which population is the closest to the Polish population. We, as transplantologists, have concluded from this that unrelated bone marrow donors from neighboring bone marrow donor registries are better, in immunogenetic terms, than donors from geographically more remote registries.

## MHC not the whole story

MHC compatibility is not the sole factor determining transplant success and tissue engraftment in the recipient. The immunological self includes also minor histocompatibility antigens (mHA). These are HLA-bound peptides derived from self proteins. T cell receptors, which are able to recognize self MHC-peptide structures, are characterized by low affinity to these structures. This ensures immunological tolerance and main-




**The genetic distance determined on the basis of the distribution of HLA-A, -B and -DRB1 alleles reflects the geographic distance between populations**

tains a pool of what are called naïve T cells. It is believed that a slight change in the sequence (e.g. a change of only a single amino acid) of the presented peptide may increase the affinity of the MHC-peptide complex for a T cell receptor and lead to T cell activation, thus inducing an immune response against a pathogen or non-self cells. Unfortunately, our knowledge of the sequences and tissue specificity of peptides relevant for transplantation is very limited.

A comprehensive study on the clinical significance of peptide differences with regard to complete HLA compatibility between the bone marrow transplant donor and recipient is the subject of a grant for which our Institute together with Warsaw Medical University is applying to the Poland's National Centre for Research and Development. We plan to carry out the study using whole exome sequencing (WES), which we hope will explain some properties of the peptide self and failure of bone marrow or haematopoietic stem cell (HSC) transplantation associated with its incompatibility.

## Innate system is also important

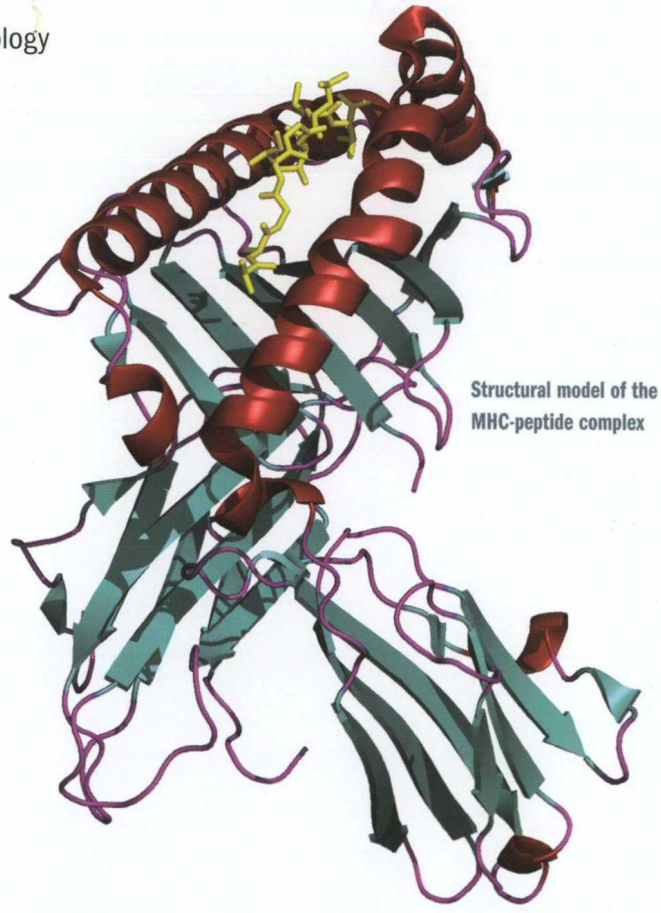
Major histocompatibility complex (MHC) together with T cell receptors are components of the acquired immune system. However, mechanisms of the innate immune system are also relevant in the recipient's immune surveillance. A significant role is played here by killer cell immunoglobulin-like receptors (KIRs), expressed on the surface of naturally cytotoxic cells, i.e. natural killer cells (NK cells). As their name suggests, NK cells can kill other cells. Nevertheless, this tendency is inhibited by KIR binding to some HLA class I molecules. In this case, HLA molecules act as the immunological self as their presence on every cell of the body protects tissues against NK cell-mediated attack. In the case of viral infection or inflammation associated with neoplastic transformation, increased expression of NK cell-activating ligant molecules in the transformed cell takes place, triggering activating NK cell receptors. Then, the activating signal dominates the inhibitory signal, and NK cells start killing such cells. The

issue of the self is one of the key research themes in immunology, also at our center. Comprehensively identifying its structures, and hence understanding its function, could boost the effectiveness of treatment of infectious, neoplastic and autoimmune diseases, improving the successful outcome of transplant treatment and lowering the incidence of some post-transplant complications.

## When conflict occurs...

Donor-recipient HLA incompatibility in haematopoietic stem cell transplantation leads to various forms of immunological conflict. If donor T cells recognize foreign HLA molecules on the recipient's tissues, most often acute graft versus host disease (aGvHD) develops. About 10% of T cells transplanted from an HLA-incompatible donor show direct reactivity with the recipient's tissues without prior immunization. The acute form of GvHD induced by this lymphocyte population destroys major immunological barriers, such as the skin, intestinal mucosa, liver, etc. This complication is associated with a high rate of mortality and requires intensive immunosuppression both for the prevention and treatment of aGVHD. This, in turn, promotes the development of infections. Subsequently, the recipient may develop chronic GvHD (cGvHD). The delay is caused by the time needed to generate a cellular immune response, probably induced by mHA incompatibility, often accompanied by HLA incompatibility. Although chronic GvHD occurs more rarely than its acute form, the former is more difficult to treat and more often leads to the recipient's death.

However, we are not helpless against these complications. Currently, about 80% of Polish patients receive a completely HLA-compatible bone marrow transplant, while as recently as 5 years ago, this percentage was about 35%. This is possible due to effective bone marrow donor recruitment programs and a certain praiseworthy altruism in Polish citizens recruited as donors. The Bone Marrow Donor Center of the Institute of Hematology and Transfusion Medicine is involved in the National Program for the Development of Transplantation Medicine, which has helped bring about an increase in the number of registered donors from 30,000 to over 700,000 in the last 6 years. To handle patients for whom there are no completely compatible donors in registries, donor selection methods with what is called "permissive mismatch," i.e. mismatch of permissible harm, are being developed.



Structural model of the MHC-peptide complex

Our initial results indicate that alongside the number of HLA mismatches (10 compatible HLA alleles out of 10 considered being seen as good, 9/10 or 8/10 as worse, fewer as even worse), the distribution of mismatches in both parental MHC supergenes, called haplotypes, is very important for HSC recipient survival. Therefore, we are currently developing a selection algorithm for selecting a partially compatible HSC donor in such a way that mismatches would affect only one of the two donor MHC haplotypes. This work accompanied by an improvement in medical procedures that prevent complications by using more effective new generation immunosuppressive agents, and monoclonal antibodies, by adjusting chemotherapeutic agents and radiation preparation regimens to the patient's condition, and through pre-emptive prevention of virus activation, antibiotic therapy or manipulation of the transplant cellular composition.

## Immunological domination

After HSC transplantation, the donor's and recipient's immune systems remain in conflict and try to dominate each other. The activity of the recipient's immune system can be substantially reduced by immunosuppressive preparation and chemotherapy, whereas the transplant's immune system cannot be inhibited in order to increase the chances of engraftment. Therefore, a considerably more frequent problem is an unfavorable immunological effect of the graft against the recipient's tissues in the form of the aforementioned GvHD. An immunological effect in the opposite direction, i.e. transplant rejection by the recipient, occurs significantly more rarely, but it may also occur due to HLA mismatch. Moreover, here medicine can minimize complications through an efficient search for completely compatible donors for a wider and wider range of patients. Progress in this area has been also made due to effective patient preparation tailored to the individual patient's condition (myeloablative and non-myeloablative conditioning), and methods developed for the prevention of transplant rejection by means of re-transplantation or infusion of a controlled dose of donor lymphocytes (donor lymphocyte infusion, DLI).

HSC transplantation is most frequently indicated for patients with malignancies such as acute or chronic leukemia, or, more rarely, lymphoma or multiple myeloma. In many cases, HSC transplantation provides a cure for these diseases due to immunological eradication of cancer cells by the donor immune cells. Notably, these desired outcomes, called the graft versus tumor (GvT) effect and adverse GvHD, are mediated by the same donor immune system and little is known about their discrimination. For the highest benefit of the patient, the later must be limited by providing properly adjusted immunosuppression and immunogenetical matching without significant loss of the former. The relapse of cancer and GvHD in the recipient are two examples of immunological conflict, of which only one (GvT) may eventually yield a positive result, which in this case means cure of malignancy.

In post-transplant immunosurveillance of cancer, the donor's NK cells also play a significant role. According to the hypothesis of missing self recognition, an absence of the recipient's HLA class I molecule, which binds to the inhibitory KIR receptor of the donor's NK cells, is favorable, as NK cells deprived of inhibition destroy a cancer cell without HLA in a more effective way. So far, this theory has been confirmed for acute myeloid leukemia. Results reported for other types of cancer have not been coherent.

Our institute is working on this issue, taking advantage of the recent discovery of donor NK cell licensing. Licensing consists in a full cytotoxic competence being attained by the donor's NK cells due to simultaneous expression of the inhibitory KIR receptor and its ligand, i.e. an HLA class I molecule. Non-licensed NK cells have a very weak effect against cancer. Our studies have not confirmed any anti-cancer effectiveness of missing KIR ligand. Thereby, we have not confirmed the theory of missing self recognition. On the contrary, we have found that in the presence of licensed NK cells in the donor, the lack of KIR ligand in the HSC transplant recipient with malignancy induced a dramatic decrease in time to relapse and the overall survival of patients. This indicates the need to avoid partially incompatible donors if the mismatch is due to the lack of the HLA class I molecule (KIR ligand) in the recipient, which is responsible for licensing the donor's NK cells. Based on these results, during immunogenetic selection we have to carefully avoid donors with that type of HLA incompatibility.

It is difficult to state whether improvement of donor selection with regard to immunogenetic compatibility or improved peri-transplantation medical procedures will have a larger impact on the improvement, safety and effectiveness of HSC transplantation as a method for cancer treatment. But one thing is certain: there is certainly no conflict between such preemptive efforts to improve selection techniques and direct medical procedures. ■

**Further reading:**

Nowak J. (2008). Role of HLA in hematopoietic SCT. *Bone Marrow Transplantation*, 42(S2), 71-76.

Nowak J, et al. (2014). Donor NK cell licensing in control of malignancy in hematopoietic stem cell transplant recipients. *American Journal of Hematology*, 89(10), 176-183.