Genetic Variability and Expression of NK Cell Receptors Belonging to the NKG2 Family and Their Ligands in Allogeneic Hematopoietic Stem Cell Transplantation Outcome

Summary

Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) is a curative procedure used as a treatment in haematological diseases or disorders, including blood cancers. In contrast to autologous, allogeneic HSCT is performed with the use of biologic material from donor specifically matched within the Major histocompatibility complex (MHC) with recipient. Despite high success rate, developing new conditioning and prophylaxis strategies, HSCT may still lead to development of some post-transplant complications. These complications include infections (bacterial, fungal and viral), graft rejection, relapse of the primary disease or Graft-verus-Host Disease(GvHD). One of the most common post-transplant complications is Cytomegalovirus (CMV) infection and GvHD, which can occur in two forms; acute and chronic. The proper reconstitution of immune system is critical for the success of HSCT.

Natural Killer (NK) cells are the first donor-derived lymphocytes, which are reconstituted in recipient organism after allogeneic HSCT. NK cells exhibit characteristics of both innate and adaptive immunity, making them a compelling subject of study. Proper functioning of NK cells is regulated by a set of activating and inhibitory receptors, which are located on their surface. One of the receptor groups is the C-type lectin-like NKG2 family members of which are inhibitory NKG2A and activating NKG2C and NKG2D. They can react with their target cells, which show surface expression of HLA-E, MICA and MICB, belonging to non-classical MHC class I molecules. Genes encoding for these molecules are localized within the MHC region on the short arm of chromosome 6. Similar to classical, non-classical MHC molecules are highly polymorphic. HLA-E molecule is an exception which polymorphism is mostly limited to two alleles (*01:01 and *01:03) present in the general population with almost 99% frequency and with a similar distribution. HLA-E is a ligand for two receptors; inhibitory NKG2A and activating NKG2C (encoding by *KLRC1* and *KLRC2* genes, respectively). Despite the structural similarity of both receptors, the HLA-E binding affinity of NKG2A is 6-fold higher than of NKG2C, which is caused by amino acid differences at positions (for NKG2C) and 167–170 (for NKG2A). The most polymorphic molecule of non-classical MHC is MICA with over 100 alleles discovered. Both MICA and MICB are ligands for activating NKG2D receptor, which presence is associated with cytolytic and cytotoxic properties of NK cells. MICA/MICB and HLA-E molecules can be detected as transmembrane proteins as well as in their soluble forms (sMICA, sMICB, sHLA-E) in the extracellular matrix.

Results obtained for this doctoral dissertation are described in three publications (two published papers, one paper currently under review). These publications aimed to describe polymorphic diversity of genes encoding for NKG2A, NKG2C and NKG2D receptors as well as their ligands HLA-E, MICA and MICB molecules. They also examined the surface expression of NKG2 receptors on NK cells and the concentration of soluble forms of their ligands in serum samples.

The first paper focused on NKG2A and NKG2C receptors and their ligand, the HLA-E molecule. Studies on genetic distribution of *NKG2A*/*KLRC1*, *NKG2C*/*KLRC2* and *HLA-E* showed that HSCT recipients diagnosed with acute myeloid leukaemia (AML, which was the most common diagnosis in our study group) the *NKG2A* rs7301582 *C* allele was more frequently present when compared with the donor group. Recipients who developed acute GvHD in more severe II-IV grades were more frequently characterised with the presence of deletion localized within gene encoding for NKG2C activating receptor when compared with individuals without or diagnosed only with mild grade I aGvHD. As for the HLA-E molecule, its donor/recipient mismatch within the rs1264457 polymorphism was associated with a higher incidence of chronic GvHD and CMV infection when compared to fully HLA-matched (10/10) donor/recipient pairs. No association between HLA-E mismatch and acute GvHD development was observed. Next, the relationship between soluble HLA-E concentration in serum collected after HSCT and risk of development of post-transplant complications was checked. Measurements showed

that recipients with chronic and acute GvHD are characterised with decreased sHLA-E level when compared with individuals free of these complications. Additionally, increased serum sHLA-E was observed in samples collected 90 days after HSCT in comparison to samples collected at day +30 after transplantation. Analysis of surface expression of NKG2C receptor on the NK cells was performed with the use of flow cytometry. The results showed increased percentage of NKG2C+ NK cells in recipients diagnosed with post-transplant CMV infection at day +60 and +90 after HSCT in relation to individuals without infection. Similarly, the percentage of NKG2A-NKG2C+ NK cell population was increased in CMV-infected individuals. Furthermore, an association between genetic polymorphisms and surface expression of NKG2C receptor was observed. Among recipients carrying at least one *NKG2A* rs7301582 *T* and with the *NKG2C* deletion the percentage of NKG2C+NK cells was decreased as compared with other recipients.

Second publication presented results of the studies on MICB molecule, which is one of the activating NKG2D receptor's ligands. Genetic variability studies showed that in both analysed polymorphisms (rs1065075 and rs3828903) the *G* allele was less frequently detected among donors for recipients who developed cGvHD. Additionally, the *MICB* rs1065075 *G* allele was less frequently present among donors for recipients with CMV infection. The same relationship was observed in the recipient group. This association was additionally confirmed by a multivariate analysis, which included recipient's age and the CMV IgG serostatus of both the donor and recipient prior to transplantation. Studies on serum sMICB was performed on samples collected 30 days after HSCT procedure. The obtained results showed that recipients who developed CMV infection or those who were diagnosed with cGvHD were characterised with increased level of serum sMICB, as compared to individuals free of these complications. Moreover, sMICB concentration could also be related with the presence of specific genetic variants. The homozygous *AA* genotype of both studied polymorphisms (rs1065075 and rs3828903) was associated with increased concentration of serum soluble MICB.

In the third, unpublished manuscript, the issues related to the MICA molecule and the activating NKG2D receptor were discussed. Studies on soluble MICA in serum collected 30 and 90 days after transplantation revealed that its concentration increased with time after HSCT. Increased level of sMICA was also detected at day +30 in recipients who were diagnosed with acute and chronic GvHD. A possible relationship between serum sMICA and *MICA* genetic variants was also observed. Recipients carrying at least one *MICA*1065075 *G* allele (genotypes *AG* and *GG*) as well as recipients whose donors were carriers of *MICA*1065075 *G* allele characterised with increased serum sMICA when compared to *AA* homozygous individuals. Among the recipients diagnosed with chronic GvHD, the NKG2D surface expression on NK cells was significantly increased at days +60 and +90 after HSCT. Furthermore, the percentage of NKG2D+ NK cells differed in recipients based on the sMICA concentration. Individuals with lower serum sMICA concentration (sMICA< median) were characterised with increased percentage of NKG2D+ NK cells 30 days after transplantation. *NKG2D*rs1049174 polymorphism showed potential prognostic properties in development of aGvHD. Genetic distribution of this SNP in recipients without or with mild grade I of aGvHD was similar to distribution in the control group (unrelated healthy volunteers from the Regional Centre of Transfusion Medicine and Blood Bank in Wroclaw, Poland). Differences were observed when the healthy individuals and recipients without or only with mild grade I were compared with recipients who developed more severe grades II-IV of aGvHD.

Additionally, this doctoral dissertation includes a review paper on the activating NKG2C receptor. The paper describes the biological and clinical functions of the receptor as well as its interactions with HLA-E molecule, which is a common ligand for both NKG2A and NKG2C receptors.

The findings presented in this series of publications which are part of this doctoral dissertation highlight the significance of genetic variability and expression of NKG2 NK cell receptors in the reconstitution of immune system in recipients after allogeneic HSCT. Presence of specific genetic variants associated with development of post-transplant complications could be used as a prognostic factors. Soluble forms of NK cell receptors' ligands could potentially be used as a biomarkers for development of post-transplant complications such as GvHD or CMV infection.