Importance of killer immunoglobulin-like receptors in allogeneic hematopoietic stem cell transplantation

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Hematopoietic stem cell transplantation is the treatment of choice for many hematologic diseases, such as multiple myeloma, bone marrow aplasia and leukemia. Human leukocyte antigen (HLA) compatibility is an important tool to prevent post-transplant complications such as graft rejection and graft-versus-host disease, but the high rates of relapse limit the survival of transplant patients. Natural Killer cells, a type of lymphocyte that is a key element in the defense against tumor cells, cells infected with viruses and intracellular microbes, have different receptors on their surfaces that regulate their cytotoxicity. Killer immunoglobulin-like receptors are the most important, interacting consistently with human leukocyte antigen class I molecules present in other cells and thus controlling the activation of natural killer cells. Several studies have shown that certain combinations of killer immunoglobulin-like receptors and human leukocyte antigens (in both donors and recipients) can affect the chances of survival of transplant patients, particularly in relation to the graft-versus-leukemia effect, which may be associated to decreased relapse rates in certain groups. This review aims to shed light on the mechanisms and effects of killer immunoglobulin-like receptors - human leukocyte antigen associations and their implications following hematopoietic stem cell transplantation, and to critically analyze the results obtained by the studies presented herein.

Keywords: *Hematopoietic stem cell/transplantation; Histocompatibility testing; Receptor KIR/immunology; HLA antigens; Killer cells, natural; Graft vs host disease.*

Introduction

Allogeneic hematopoietic stem cell transplantation (HSCT) is the treatment of choice for many hematologic diseases. However, chronic graft-versus-host disease (GvHD) and relapse are still the main obstacles to the success of this therapy.

Currently, a number of possible interventions are being studied in different parts of the world to reduce these problems. The use of Natural Killer (NK) cells is one such alternative because there is the possibility of leukemic cell lysis by NK cells from the donor.

These studies may lead to important changes in the strategy of selecting unrelated donors in HSCT, thereby increasing not only the survival of transplant recipients, but also providing an improved quality of life of patients after transplantation.

Natural Killer cells

NK cells were characterized over 30 years ago as a cytotoxic effector of the innate immune system. Recognized as a subtype of lymphocytes that have cytoplasmic granulation, NK cells are larger than conventional lymphocytes and are found in the peripheral circulation and in various tissues and organs such as the bone marrow, spleen, lymph nodes, liver, intestine and placenta. Approximately 15% of all circulating lymphocytes are NK cells,[1] and these constitute the first line of defense against pathogens such as intracellular bacteria, parasites and, in particular, viruses, in addition to preventing the formation of tumors. They act by releasing cytokines or by lysis of the target cell. Many of these substances initiate and maintain adaptive immune responses; cell lysis is mediated by apoptosis involving granule exocytosis (perforin and granzymes) and Fas/FasL (Fas Ligand) binding.[2]

Recent studies have increased our understanding of how NK cells recognize target cells. The response of these cells is intimately involved in the interaction with Human leukocyte antigen (HLA) class I molecules present on target cells. Two large families of NK cell surface receptors have been identified – CD94/NKG2 and killer immunoglobulin-like receptors (KIRs).[3] As KIRs are highly polymorphic and interact with a larger number of HLA class I molecules, they have become extremely important in studies on susceptibility to infections, on HSCT and on cancer.

The group of KIR genes comprises a region of approximately 150 Kb in the leukocyte receptor complex (LRC) on chromosome 19q13.4. KIRs are members of a group of regulatory molecules on the surface of NK cells, in subgroups of Tγδ+ lymphocytes, effector Tαß+ lymphocytes and memory lymphocytes.[4]

The KIR family includes activating and inhibitory molecules. Inhibitory KIRs (2DL and 3DL) have a long cytoplasmic tail containing tyrosine-based inhibitory motifs (ITIMs) that trigger inhibitory events of cytotoxicity. In contrast, activating KIRs (2DS and 3DS) interact with the DAP12 molecule, which has tyrosine-based activation motifs (ITAMs) that cause a cascade that results in an increase in cytoplasmic granulation and the production of cytokines and chemokines, thereby initiating immune response.[5]

KIRs are the main functional regulators of NK cells. The balance between activation and inhibition of NK cells occurs through the binding of KIRs with HLA class I molecules present in all nucleated cells of an individual. The different forms of these receptors are specific to certain HLA molecules and some of these KIR-ligand pairs are already known. The KIR2DL4, for example, specificity binds to the HLA-G molecule,[6] while the KIR3DL1 receptor binds to a subset of HLA molecules with the Bw4 epitope, present in approximately one third of all HLA-B molecules. The KIR3DS1 is highly homologous with 3DL1 and seems to share the Bw4 epitope as ligand, although this needs to be experimentally verified. The KIR3DL2 receptor is still being discussed, but studies suggest that HLA-A3 and HLA-A11 perform this role.[7]

Most KIRs bind to HLA-C molecules. It is worth remembering the importance of the dimorphism of amino acids, such as residue 80 of α-helix-1, in the definition of this HLA receptor. On this basis, HLA-C alleles may be defined as "Group 1" or "Group 2". The KIR2DL2, 2DL3 and 2DS2 - HLA-C*01, *03, *07 and *08 alleles are included in "Group 1". And "Group 2" consists in the KIR2DL1 and 2DS1 - HLA-C*02, *04, *05 and *06 alleles.[8] Finally, evidence suggests that HLA-CW4 is a receptor for KIR2DS4.[9]

Allogeneic hematopoietic stem cell transplantation

Allogeneic HSCT is the treatment of choice for many hematologic diseases. The outcome of HSCT is dependent on several factors which include disease stage at the time of transplant, the conditioning regimen, source of cells, the degree of HLA matching between donor and recipient and the development of chronic GvHD. Recent studies have indicated that other potential factors could also influence the course of the transplant.[10-12]

The first attempts to investigate the role of NK cells in HSCT were carried out in haploidentical transplants (only one compatible HLA haplotype). The results of haploidentical transplants in the early 1990s were disappointing mainly due to the high incidence of mortality, often caused by alloreactive T cells of the donor against recipient (often leading to fatal GvHD) or recipient against donor (rejection). The introduction of concepts such as 'missing self' by NK cells in the clinical practice has led to innovative approaches in the treatment of hematologic malignancies.[13]

Killer immunoglobulin-like receptors and haploidentical hematopoietic stem cell transplantation

The chance that a donor presents a HLA combination identical to a sibling recipient is 25%. In many cases, there is no compatibility in the family and an unrelated donor is not always found. In this situation it is possible to perform HSCT with a family member who has only one identical HLA haplotype (haploidentical). Thus, this partial compatibility can be found in both parents and in 50% of siblings. In haploidentical transplantation, the presence of T cells in the graft means strong reactivity of these cells against the alloantigens of the recipient. Alternatively, T-cell depletion reduces graft-versus-host reactions, but allows a higher incidence of leukemic relapse due to a reduced graft-versus-leukemia effect.[14] In theory, NK cells present in the graft might overcome this lack of alloreactivity of the graft against leukemia, as long as they are not inhibited by their corresponding HLA ligands in the recipient.

One of the first studies that evaluated the alloreactivity effect of NK cells by KIR-HLA mismatch in HSCT involving haploidentical pairs enrolled 60 patients with acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML) and chronic myeloid leukemia (CML).[15] The presence of HLA incompatibility may favor both graft versus host and host versus graft alloreactivity mediated by T cells. Thus, T cell depletion was performed. The results showed that patients with myeloid leukemias suffered alloreactivity of NK cells from the donor, following the rules of KIR-HLA mismatch. This study brought to light a new aspect of haploidentical HSCT: lysis of residual leukemic cells in the recipient by allogeneic NK cells.

For a more comprehensive investigation of this effect, the same group increased the number of patients in a new study and included models of NK cell alloreactivity in mice.[16] Thirty-four haploidentical transplants with KIR-HLA mismatches and 58 KIR-HLA matched haploidentical transplants were analyzed. In AML patients, transplantation with alloreactive NK cells fully protected the group against rejection, GvHD and leukemic relapse. The probability of disease-free survival at 5 years was 60% in this group in contrast to 5% in the group without alloreactivity. Multivariate analysis, which considered the conditioning regimen, the number of hematopoietic stem cells and T cells in the graft and disease stage at transplantation, showed that graft versus host KIR-HLA incompatibility was an independent factor for increased survival. This effect was not observed in ALL patients.

From these observations, alloreactive NK cells were infused into mice previously conditioned to develop human AML. The mice that received non-alloreactive NK cells died within three weeks. However, those that received NK cells with KIR-HLA mismatches survived until the day of sacrifice (120 days). These observations showed that the alloreactivity generated by NK cells is associated with a marked graft-versus-leukemia effect, and a total control of rejection. Other data suggest that alloreactive NK cells accelerate the elimination of antigen-presenting cells (APCs) from bone marrow, spleen and intestine, suggesting a mechanism that prevents GvHD by the elimination of APCs.

Contradictory results were demonstrated for 62 patients who also received haploidentical donor grafts.[17] It was found that the alloreactivity of NK cells was associated with a high incidence of GvHD. What both studies emphasized was the difference in pre-transplant protocols. The contradictory results of these two studies may be related to the lack of T cell depletion in recipients or by the presence of a significantly greater number of these cells in the graft received by patients in one study[17] compared to the other.[16] Thus, the beneficial role of NK cell alloreactivity may be masked by the presence of T cells, suggesting that the effects of the KIR-HLA mismatch depend on specific conditioning protocols.

Experimentally, some authors demonstrated that the infusion of haploidentical donor NK cells, in patients who received conditioning regimens with high doses of cyclosporine and fludarabine, was associated with increased levels of IL-15, essential in the final differentiation of $CD34^+$ hematopoietic stem cells into NK cells.[18] Furthermore, there was a higher rate of complete remission in these patients, especially those receiving infusions of NK cells with KIR-HLA mismatches, and possibly presenting a graft-versus-leukemia effect.

In a study, which analyzed 137 patients with severe combined immunodeficiency (SCID), 124 received haploidentical HSCT.[19] SCID is a fatal genetic disorder that combines lymphopenia with the absence of T cells, increasing susceptibility to infection. Although the incidence and severity of GvHD were not different, analysis of survival at five years showed that the HLA-KIR mismatch might favor survival but without statistical significance. The authors suggested that NK cell alloreactivity may not play a fundamental role in HSCT of patients with SCID.

Another study evaluated 109 AML patients, 57 patients reported in 2002 and over 52 since then.[20] When the alloreactivity of NK cells was considered, event free survival was better for remission patients and even for relapsed patients. The study demonstrated that this strategy not only prevents GvHD, but also provides a pronounced graft-versus-leukemia effect that significantly increases the survival of transplanted patients. According to the authors, alloreactivity of NK cells from the donor against the recipient emerges as a crucial factor in promoting improved survival in haploidentical HSCT.

If incorporated as a selection criterion, the chance of finding a donor with alloreactive NK cells rises from 33% to 49%,[21] which would represent a substantial gain for recipients. Another advantage is the increased availability of haploidentical donor relatives may reduce delay to transplant.

Killer immunoglobulin-like receptors and related hematopoietic stem cell transplantation

The ideal for patients who require HSCT donors is to find an HLA identical relative. This type of transplantation usually provides quick engraftment, a decreased rate of GvHD and greater success in immune reconstitution. As the chance of finding an HLA identical related donor is 25% in the clinical practice about three quarters of recipients will need haploidentical transplantations or transplants using volunteer donors.[21]

In 2004, one study carried out KIR-HLA genotyping of 220 related HLA identical donor-recipient pairs (112 for myeloid diseases and 108 lymphoid diseases).[22] For patients with myeloid diseases, survival was lower in those homozygous for Group 2 (C2) HLA-C compared to patients with Group 1 (C1). This effect was observed only when the donor had the KIR2DS2 gene. As KIR2DS2 is in strong linkage disequilibrium with KIR2DL2 (receptor inhibited by C1), this would indirectly indicate lower survival in patients who do not have the receptor for KIR2DL2, an opposite result to the model in which this lack of inhibition would result in NK cell alloreactivity with a consequent elimination of residual leukemic cells.[23]

In 178 patients with AML, CML, ALL and primary myelodysplastic syndrome (MDS) who received HSCT with T cell depletion from HLA-identical related donors, some authors observed that the disease-free survival was significantly higher in patients with AML and MDS that did not have the HLA ligand for the inhibitory KIR of the donor.[24] Moreover, the relapse rate was lower in these individuals, which may be related to higher survival rates. The results differ from a study in which T cell depletion was not performed.[22]

In another study[25] involving 83 patients with different types of hematologic malignancies who received HSCT from related HLA-identical donors without T cell depletion, a high relapse rate was found when high numbers of activating KIRs were present in both the patient and donor. According to the authors, a consequence of this finding may be an increased alloreactivity of the host against graft, impairing the response of donor cells resulting in an insufficient graft-versus-leukemia effect and increased risk of leukemic relapse.

Killer immunoglobulin-like receptors and unrelated hematopoietic stem cell transplantation

Patients in need of HSCT who do not have an HLA-identical donor in the family may benefit from unrelated HLA compatible donors. The advent of high resolution HLA

genotyping and the identification of new alleles, as well as advances in the prevention and treatment of GvHD have improved the survival rate of transplanted patients. Consequently, Registers of volunteer bone marrow donors have been organized in several countries. In 2010, the number of volunteer donors on the register in Brazil was approximately 1,400,000.[26]

The role of the KIR-HLA mismatch in unrelated donors was first explored in 2002 in a study that examined 175 unrelated pairs with one HLA mismatch at the class I locus.[27] The groups, divided between those with or without KIR-HLA mismatches, included patients with different hematological diseases. The most intriguing result was that in patients with myeloid disease characteristics, the five-year survival was lower in the KIR-HLA mismatch group.

The advantage of the alloreactivity of NK cells was re-confirmed in a study of unrelated donors.[28] Conditioning was standard, including T-cell depletion using ATG (anti-thymocyte immunoglobulin). The disease-free survival of KIR-HLA mismatch patients was significantly higher compared to patients without mismatches. The benefit was even greater when patients with myeloid diseases were analyzed separately, none had leukemic relapse or died of transplant-related complications. Due to the potent immunosuppression provided by ATG, engraftment may have been improved, and consequently a more rapid recovery of alloreactive NK cells of the graft may have occurred.

Contradictory results were presented by a later study of patients with AML, CML and MDS who received either partially matched HLA grafts or from unrelated HLA-identical donors.[29] An increased likelihood of leukemic relapse was found for recipients with HLA-KIR mismatches. In response to these findings, some differences between the two studies must be mentioned, such as the mean age of patients, the conditioning protocols and the stem cell source. The first used predominantly peripheral hematopoietic stem cells and the mean age of patients was higher, indicating an advanced stage of disease in these recipients, favoring leukemic relapse.

In 2005, a study evaluated 374 patients with myeloid leukemia and found decreased rates of leukemic relapse in donor-recipient pairs with KIR-HLA mismatches however graft failure rates were higher.[30] There were no significant differences in disease-free survival or transplant-related mortality.

Another group, in an analysis of 104 patients who were submitted to HSCT with unrelated donors, found increased rates of rejection in patients with alloreactive NK cells against the graft, due to lysis of donor cells by the recipient's NK cells.[31] Acute Grade III and IV GvHD was more frequent in transplants with KIR-HLA mismatches, a result similar to other studies involving protocols without extensive T cell depletion. Interestingly, the presence of a high number of activating or inhibitory KIR genes in donors had a protective effect against GvHD and gave a higher survival rate.

Two large retrospective multicenter studies were then performed. In the first, which enrolled 1770 patients, there was a decreased leukemic relapse rate in recipients with KIR-HLA mismatches.[32] The second, however, found that, in an analysis involving 1571 recipients, incompatibility is disadvantageous.[33] Contradictory results may occur in surveys of this size; these are very heterogeneous studies with differences in conditioning protocols, immunosuppression and transplantation techniques.

Most studies conducted on HSCT focus on the effect of the lack of inhibitory KIRs by their HLA class I pair, and the role of activating KIRs remains largely undefined. The results of a study in 2006 of 25 patients who received unrelated donor HSCT (23 HLA identical) showed a higher mortality risk and incidence of GvHD when the gene for the activating receptor, KIR2DS2, was present in the donor. The presence of KIR2DS2 might increase the alloreactivity of donor cells (especially for certain subgroups of T cells that express KIRs) and result in severe GvHD.[34]

Conclusions

It is noteworthy that many studies did not perform KIR genotyping, but used an algorithm to predict KIR-HLA mismatches. Not always the presence of the corresponding HLA ligand means inhibition as the KIR gene may be absent. Another factor that may be related to the survival of these patients is the presence of activating KIRs, which is information only available from genotyping. High resolution sequencing of KIR genes is also proving useful to identify the real alleles involved in the rejection process, GvHD and relapse, all factors that affect survival rates.

More recent studies have been developed to establish trials that can provide information regarding to the alloreactivity of 'donor-recipient' pairs, such as NK cell crossmatch, which, by means of serological testing, can check the possibility of leukemic cell lysis by donor NK cells.[35] These studies may lead to important changes in the unrelated donor selection strategy for hematopoietic stem cell transplantation, in an attempt not only to increase survival of transplant recipients and but also to provide a better quality of life for patients after HSCT.

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