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NK cell alloreactivity and allogeneic hematopoietic stem cell transplantation

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Abstract

As only 60% of leukaemia patients find a matched donor, the Perugia Bone Marrow Transplant Centre developed transplantation from HLA haplotype-mismatched family donors to provide a cure for more patients [F. Aversa, A. Tabilio, A. Terenzi, et al., Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum, Blood 84 (1994) 3948–3955] [F. Aversa, A. Tabilio, A. Velardi, et al., Treatment of high-risk acute leukemia with T-cell-depleted stem cells from related donors with one fully mismatched HLA haplotype, N. Engl. J. Med. 339 (1998) 1186–1193] [F. Aversa, A. Terenzi, A. Tabilio, et al., Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse, J. Clin. Oncol. 23 (2005) 3447–3454]. HLA-mismatches trigger donor vs. recipient NK cell alloreactivity which improves engraftment, protects from GvHD and reduces relapse in AML patients [L. Ruggeri, M. Capanni, E. Urbani, et al., Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants, Science 295 (2002) 2097–2100], [L. Ruggeri, A. Mancusi, M. Capanni, E. Urbani, A. Carotti, T. Aloisi, M. Stern, D. Pende, K. Perruccio, E. Burchielli, F. Topini, E. Bianchi, F. Aversa, M.F. Martelli, A. Velardi, Donor natural killer cell allorecognition of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value, Blood, in press]. We are using murine transplant models to determine whether NK cell alloreactivity can be exploited to reduce transplant-related mortality (TRM) which remains a major issue. Data from these on-going studies show pre-transplant infusion of alloreactive NK cells: (1) ablates AML cells, (2) kills recipient T cells, permitting a reduced toxicity conditioning regimen, and (3) ablates the recipient dendritic cells (DCs) which trigger GvHD, thus protecting from GvHD while permitting a higher T cell content in the graft. We are designing a clinical haploidentical transplant trial using alloreactive NK cells in the conditioning regimen, with the aim of reducing TRM and improving outcomes and overall survival.

Keywords: Acute leukaemia; NK cells; Alloreactivity; HLA-mismatches

Background

Donor human leukocyte antigen (HLA) incompatibility increases the risks of graft failure, graft versus host disease (GvHD) and transplant-related mortality (TRM). Early T-replete bone marrow transplants found donor incompatibility for two or three HLA antigens was a formidable barrier, as GvHD could not be controlled with post-transplant immunosuppression and very few patients survived. In patients with leukaemia, T-cell-depleted bone marrow grafts reduced the incidence of acute and chronic GvHD, but the rates of graft failure and leukemia relapse rose sharply and were associated with impaired immune reconstitution and no advantage in survival. In murine models, Reisner's group [6] showed that hematopoietic stem cell dose escalation was a viable strategy for overcoming the HLA incompatibility barrier of anti-donor cytotoxic T-lymphocyte precursors (CTL-p) which survive supralethal conditioning.

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Once recombinant human granulocyte colony-stimulating factor (G-CSF) was used to mobilize peripheral blood progenitor cells and increase the number of donor stem cells to the equivalent of what had crossed the histocompatibility barriers in mice, extensively T-cell-depleted mismatched HSCT engrafted successfully in acute leukaemia patients [1–3].

The mismatched transplant relies for its success on the combined action of high-intensity conditioning regimens to reduce the leukemia burden to a minimum and achieve maximum immunosuppression, a "megadose" of hematopoietic cells to ensure engraftment across the HLA barrier; extensive T-cell depletion of the graft to prevent GvHD and no post-transplant immunosuppression to ensure immune reconstitution is unhindered. Today, transplantation from a full HLA haplotype mismatched family member is a viable option for patients with acute leukemia at high risk of relapse who urgently need a transplant and do not have a matched unrelated donor. Haploidentical donors range from siblings, parents and recipient's children to cousins, nephews and aunts and uncles, offering the advantages of immediate donor availability and a second transplant should the first fail.

Extensive T-cell depletion of the graft might be expected to result in a weak or no graft versus leukemia (GvL) effect which is conventionally achieved through T-cell-mediated alloreactions directed against histocompatibility antigens displayed on recipient leukemia cells. However, another cell of the immune system influences outcomes of hematopoietic cell transplantation in a surprisingly favourable way. In haploidentical transplants that are KIR ligand-mismatched in the graft-versus-host (GvH) direction, functional donor NK cells that express as their sole inhibitory receptor for self, a KIR for the HLA class I group which is absent in the recipient, sense the missing expression of the self class I ligand on allogeneic targets and mediate alloreactions ("missing self" recognition) [4,5,7–10] (Fig. 1).

Most individuals can exert NK cell alloreactions as they possess a full complement of inhibitory *KIR* genes. The KIR2DL2 and/or KIR2DL3 receptors for HLA-C group 1 are present in all; the KIR2DL1 receptor for HLA-C group 2 is found in 97% and the KIR3DL1 receptor for HLA-Bw4 alleles is found in ~90% [11–14]. Unlike inhibitory KIRs, activating KIRs exhibit extensive variation in gene number and content, which leads to heterogeneity within the general population and diverse ethnic groups [15]. Indeed, activating KIRs may not even be present in approximately 25% of Caucasians who are homozygous for the so-called group A *KIR* gene haplotypes which contain inhibitory *KIR* genes and the KIR2DS4-activating *KIR* gene (encoding for a non-functional protein in 2/3 of individuals). On the other hand, 75% of Caucasians are either heterozygous or homozygous for B haplotypes which carry not only inhibitory *KIR* genes but also various combinations of activating *KIR* genes (KIR2DS1-2-3-5 and KIR3DS1) (Fig. 2).

In vitro data show several hematological malignancies are susceptible to alloreactive NK killing. Alloreactive NK cells kill acute myeloid leukemia, chronic myeloid leukemia, T-cell acute lymphoblastic leukemia, chronic lymphocytic leukemia, non-Hodgkin's lymphoma and multiple myeloma cells [16]. Table 1 shows malignancies of lympho-hematopoietic lineage tested for alloreactive NK cell killing. Alloreactive NK cells exert significant cytotoxicity against melanoma and renal cell carcinoma cell lines [17]. In a pre-clinical model, transfer of human alloreactive NK cells eradicate engrafted human AML in non-obese diabetic (NOD)/SCID mice [4,17]. Enhanced cytotoxicity of allogeneic NK cells with killer immunoglobulin-like receptor ligand incompatibility against melanoma and renal cell carcinoma cells.

NK cell alloreactivity in haploidentical transplantation

In a limited series of haploidentical transplants (57 acute myeloid leukemia (AML) patients, 20 of whom were transplanted from natural killer (NK) alloreactive donors), donor-versus-recipient NK cell alloreactivity reduced the risk of leukemia relapse, did not cause GvHD and markedly improved event-free survival (EFS) [4]. In an update of outcomes in these patients together with another cohort of 55 more recent haploidentical transplants [5], primary engraftment was obtained in 103/112 patients. Rejection was reversed in 4/9 patients by a second haploidentical transplant from the same or other haploidentical donor. Thus, 107/112 evaluable patients engrafted with no late rejection and full donor-type chimerism. Acute ≥grade II GvHD developed in 11/112 patients. Relapses occurred in 28/112 patients. Deaths in remission were 48/112 (43%) with infectious deaths accounting for 42 of the 48. Event-free survivors were 36/112, 13 in the early series [4] and 23 in the later, at a median follow-up of 5.07 years (range 0.95–13.22).

NK cell alloreactivity had no significant impact on rejection or ≥grade II acute GvHD The incidence of relapse was similar

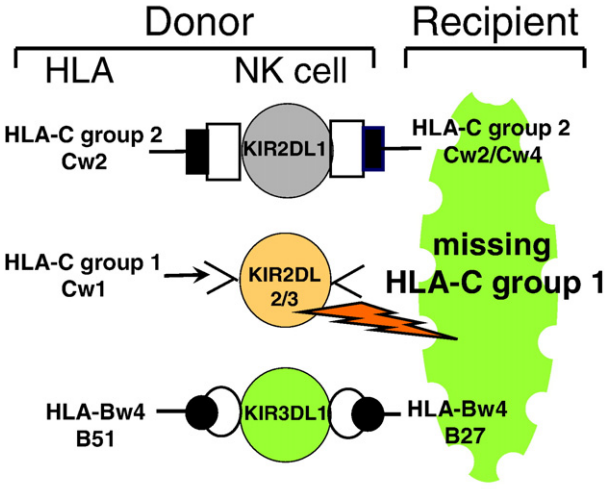

Fig. 1. Donor versus recipient NK cell alloreactivity: NK cell alloreactions are generated between donors and recipients who are KIR ligand mismatched in the GvH direction. Donor NK cells expressing, as their only inhibitory receptor for self HLA, a KIR for the class I group which is absent in the recipient, sense the missing expression of the self class I ligand on allogeneic targets and mediate alloreactions. In this example, a donor NK cell expressing KIR2DL2/3, inhibiting receptor for the self HLA-C group 1 allele, does not find this allele group in the recipient and is activated to kill the recipient target.