REVIEW

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Impact of fetal-maternal tolerance in hematopoietic stem cell transplantation

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Abstract

Allogeneic hematopoietic stem cell transplantation (HSCT) is known to cure various hematological disorders; however, its widespread use is limited due to a lack of histocompatible donors. Reciprocal cell traffic between the mother and fetus during pregnancy gives rise to postpartum fetal-maternal lymphohematopoietic microchimerism, which is frequently detected in the blood or tissue of healthy individuals. Studies in clinical and experimental transplantation provide evidence that exposure to non-inherited maternal antigens (NIMAs) during pregnancy may result in long-lasting fetomaternal microchimerism and tolerance induction. Studies of HLA-mismatched HSCT have suggested a relatively lower incidence of severe graft-versus-host disease (GVHD) after transplantation from a NIMA-mismatched donor. Studies using a mouse model have also demonstrated a "child-to-mother" bone marrow transplantation from an NIMA-exposed donor to reduce the morbidity and mortality of GVHD in an antigen-specific manner while preserving the graft-versus-leukemia effects and favoring the immune reconstitution, thus resulting in a marked improvement in outcome after HSCT. Prospective clinical studies are therefore warranted to confirm these beneficial effects of fetal-maternal tolerance in allogeneic HSCT.

Key words: HSCT, GVHD, non-inherited maternal antigen.

Abbreviations: HSCT – hematopoietic stem cell transplantation, GVHD – graft-versus-host disease, NIMA – non-inherited maternal antigen, APCs – antigen-presenting cells, NK – natural killer, IPAs – inherited paternal HLA antigens, GVL – graft-versus-leukemia, mHAs – minor histocompatibility antigens, CTL – cytotoxic T lymphocyte.

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INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative modality in a substantial number of patients with hematological malignancies, bone marrow failure, immunodeficiency syndromes, and certain congenital metabolic disorders. A growing body of evidence suggests that allogeneic HSCT is also useful for the treatment of other diseases, including solid tumors and autoimmune diseases [11, 13]. However, the widespread application of HSCT is limited because only 25–30% of potential recipients have an HLA-matched sibling. For these patients, allogeneic HSCT from an HLA-mismatched relative donor is complicated by a high incidence of severe graft-versus-host disease (GVHD).

GVHD is the most important complication of allogeneic HSCT. The pathophysiology of acute GVHD is complex, involving 1) donor T cell responses to the host alloantigens expressed by host antigen-presenting cells (APCs) activated by conditioning regimens (i.e. total body irradiation and/or chemotherapy) and 2) dysregulation of inflammatory cytokine cascades, leading to further T cell expansion and induction of cytotoxic T cell responses [59, 68, 88]. HLA disparity between the donor and recipient is the most critical factor that governs the severity of GVHD [6]. HSCT from a haplo-identical related donor has been evaluated over the past 2–3

decades as an alternative transplant option for patients who do not have an HLA-identical related donor. The advantages of haplo-identical HSCT are that nearly all patients have an immediately available donor among their family members. The disadvantages are the immunological consequences of crossing the major histocompatibility barrier, namely GVHD and graft rejection. While graft rejection can be almost always overcome with a very intensive conditioning regimen and infusion of a large number of stem cells (i.e. granulocyte-colony stimulating factor-mobilized peripheral blood stem cells) [5], severe and lethal GVHD remains a major obstacle to the success of haplo-identical HSCT. Therefore, it would be beneficial if we could determine certain permissible HLA mismatches that are less immunogenic to the recipients in the context of GVHD.

FETAL-MATERNAL TOLERANCE

Several decades ago, a remarkable discovery was made; most twin cattle were born with a stable mixed chimerism of each other's red cells [51]. Billingham et al. [7] then showed that injection of fetuses with allogeneic splenocytes allowed the acceptance of later skin grafts from the same donor, but not from other strains in mice. These results provided evidence that the exposure of neonates to allogeneic cells induced a long-lasting tolerance specific to the alloantigens of the cell donor. This phenomenon is now referred to as neonatal tolerance.

Pregnancy has to tolerate the persistence of paternal alloantigens. The fetal cells are continuously exposed to the humoral and cellular components of the maternal immune system present in the maternal blood that circulate in the intervillous space and in the decidual vessels. However, the fetus is able to evade a maternal immune attack during pregnancy. Interestingly, the maternal immune system can specifically tolerate the engraftment of paternally derived tumor cells [66]. The placenta has a unique structural organization that allows fetal cells expressing paternal alloantigens to establish a peaceful cohabitation with the maternal immune system. While the precise cellular interactions and mechanisms involved in such maternal lymphocyte tolerance have yet to be evaluated, some immunological mechanisms are now beginning to be uncovered. Fetal trophoblasts express HLA-G molecules, which suppress the activation of natural killer (NK) cells and $CD8^+$ T cells [19, 54]. These cells also express Fas ligands, which lyse activated maternal lymphocytes expressing Fas [25, 73]. The roles of the tumor necrosis factor-related apoptosis-inducing ligand-receptor and the programmed death 1 ligand in the suppression of maternal lymphocytes during pregnancy have been documented [21, 52]. Indoleamine 2,3-dioxygenase has also been shown to protect allogeneic concepti from maternal T cell-mediated immunity [40, 42]. The expression of complement regulatory protein, crry, in the placenta also promotes fetomaternal tolerance [83]. Finally, a recent study in mice demonstrated that $CD4^+CD25^+$ regulatory T cells are required for the maternal immune system to tolerate the fetal allograft [2, 15]. The enhancement of regulatory T cell function may thus contribute to a spontaneous amelioration of autoimmune diseases during pregnancy. $CD4^+CD25^+$ cells also contribute to the maintenance of neonatal tolerance by preventing the development of T cell alloreactivity [18].

FETAL-MATERNAL TRANSMISSION OF HEMATOPOIETIC CELLS

The transplacental transmission of hematopoietic cells between fetus and mother can lead to persistent microchimerism. Maternal cells and DNA can be detected for a long time in peripheral blood or various tissues such as skin, liver, and thyroid gland of offspring both in humans and in mice [3, 22, 57, 61]. Fetal cells and DNA circulate in the blood of pregnant mice [34, 36]. A murine experiment to track maternal green-fluorescent protein-positive cells showed that maternal T, NK, and B cells were persistently detected in the bone marrow and thymus of the offspring both in allogeneic and out-bred crosses [77]. Recently, granulocyte-colony stimulating factor-mobilized peripheral blood stem cell grafts obtained from some female donors have been reported to contain a significant amount of male DNA, presumed to be of fetal origin [1]. Breast-feeding in the neonatal period may also contribute to a build-up of the maternal microchimerism in offspring because breast-milk is rich in maternal MHC antigens in both soluble and cellular forms [3, 41, 78].

MICROCHIMERISM AND TRANSPLANTATION TOLERANCE

Passenger leukocytes from the allograft can also lead to a persistence of hematopoietic microchimerism after solid organ transplantation. In recipients of hepatic and renal transplants, a very low level of donor microchimerism is often found due to the trafficking of passenger leukocytes in long-term allograft recipients who require minimal immunosuppressive therapy. It was thus initially assumed that hematopoietic microchimerism plays a role in tolerance induction [63]. Later studies, however, failed to confirm this association in clinical and experimental transplantation [23, 82]. Interestingly, recent experimental studies have suggested that passenger leukocytes were involved in the induction phase of allograft acceptance and microchimerism in the thymus, but not in the blood, and were also associated with allograft survival [37, 47]. These results suggest that microchimerism may play a role in allograft survival, but persistence of peripheral microchimerism is not required. On the other hand, many investigators have suggested an association between long-term fetal-

maternal microchimerism and the development of autoimmune diseases including systemic screlosis, primary biliary cirrhosis, biliary atresia, thyroiditis, and juvenile inflammatory myopathies [4, 44, 45, 62, 64]. However, it is difficult to establish a precise association between them [30, 35, 43], because fetal and maternal microchimerism is often found even in immunocompetent individuals without any manifestations of autoimmune diseases and in healthy women with a history of an uncomplicated delivery. In addition, it has been suggested that passenger fetal cells may contribute to maternal tissue repair in both humans and rodents [33, 48, 79].

In contrast to minimal mixed chimerism after solid organ transplantation, high levels of residual host hematopoietic cells could persist after allogeneic HSCT following a non-myeloablative conditioning regimen. This so-called "macrochimerism" can be induced in humans and animals by administrating allogeneic hematopoietic stem cells following treatment with non-myeloablative regimens and it is associated with the induction of central tolerance because its leads to intrathymic clonal deletion of all newly developing donor reactive T cells [14, 39, 65]. Clinical results of non-myeloablative HSCT demonstrated the establishment of mostly transient mixed hematopoietic chimerism and a reduced incidence of GVHD compared with myeloablative HSCT, which causes full donor chimerism [60].

FETAL-MATERNAL TOLERANCE AND HSCT

The Fig. 1 shows a schematic drawing of one-haplo-identical HSCT from a non-inherited maternal antigen (NIMA)-mismatched donor. The possible role of long-term fetal-maternal microchimerism as an indicator of an acquired form of feto-maternal tolerance was first proposed in a series of case reports [26]. An infusion of peripheral blood stem cells to a mother from her HLA-haplo-identical daughter without a pretransplant conditioning regimen resulted in a dramatic regression of thymic carcinoma without any signs of GVHD [69]. Donor microchimerism was detected both before and long after the infusion in the recipient, thus prompting the authors to speculate that the mother was tolerant to paternal alloantigens on the daughter's cells and thus the infused daughter's effector lymphocytes could survive longer in the face of the maternal immune system. Ochiai et al. [50] reported a successful haplo-identical, three-loci-mismatched HSCT from a microchimeric mother without severe acute GVHD. This approach was soon found to be feasible in several groups [58, 84, 85]. More evidence was obtained in a study of patients receiving a haplo-identical HSCT from the International Bone Marrow Transplant Registry: HSCT from a non-inherited maternal HLA antigen-mismatched sibling results in a lower incidence of acute GVHD than that from a non-inherited paternal antigen-mismatched sibling donor [75]. Furthermore, although rejection is a serious problem in haplo-identical HSCT, successful engraftment has been reported even after non-myeloablative conditioning HSCT from an inherited paternal HLA antigen (IPA)/NIMA-mismatched donor in patients with hematological malignancies, aplastic anemia, and renal cell carcinoma [49, 56, 58, 72]. These results suggest that haplo-identical stem cell transplantation (SCT) based on feto-maternal microchimerism is therefore at least feasible.

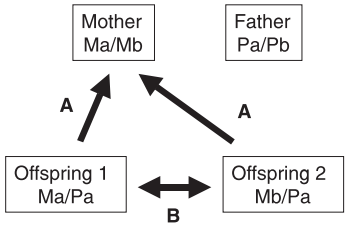

Fig. 1. A schematic drawing of one-haplo-identical HSCT from an NIMA-mismatched donor. **A** – HSCT from offspring to mother: the GVH reaction is directed against NIMAs. **B** – HSCT between NIMA-mismatched siblings who shared IPAs (Pa).

Based on these promising results, we analyzed the clinical outcomes of T cell-replete allogeneic SCT for advanced hematological malignancies from a two- to three-loci HLA-mismatched familial donor in the context of mismatch for NIMAs or IPAs in a nationwide registry study of selected cohorts of patients [27]. This analysis was confined to T cell-replete HSCT from either maternal donors who had IPA-bearing microchimeric cells or offspring/sibling donors who had NIMA--bearing microchimeric cells. Irrespective of the multiple HLA disparities between the donor and recipient, 15 of 34 evaluable patients did not develop acute GVHD of grade II or more, but severe grade III or IV acute GVHD was reported in 8 patients, thus indicating the presence of fetal or maternal microchimerism itself is not a hallmark of IPA/NIMA-specific tolerance. Importantly, the incidence of severe GVHD was significantly less in the recipients of a NIMA-mismatched donor than those of an IPA-mismatched donor. Furthermore, a multivariate analysis showed that IPA mismatch in the GVH direction has a higher risk for developing severe acute GVHD when compared with mismatches for NIMA, thus suggesting the differential immunogenecity between IPA and NIMA. However, these results should be interpreted with caution due to the small size of this study.

CELLULAR MECHANISMS OF FETAL-MATERNAL TOLERANCE

These studies in clinical transplantation suggest a tolerogenic NIMA effect, but IPA exposure does not induce tolerance. Similarly, T cells isolated from an NIMA-exposed child are hyporesponsive to the corre-

sponding antigens *in vitro*, whereas the responses of T cells from an IPA-exposed mother toward the corresponding antigens are controversial [9, 71]. In order to test the hypothesis that fetal-maternal antigen exposure can induce transplant tolerance and also to elucidate the cellular and molecular mechanisms of fetal-maternal tolerance, animal models have been developed using the F1×P back-cross breeding schema of Zhang and Miller [87]. To create NIMA-exposed mice, a B6 male and a B6D2F1 female are mated to generate H-$2^{b/b}$ offspring that are exposed to NIMA-H-2^{d} *in utero*. The controls are H-$2^{b/b}$ offspring of a B6 mother and a B6D2F1 father and are NIMA-H-2^{d} unexposed. Using this model system, Andrassy et al. [3] showed the precursor frequency of NIMA-reactive T cells to be remarkably diminished in NIMA-exposed mice. We also confirmed that T cells isolated from NIMA-exposed mice are hyporesponsive to the corresponding antigens *in vitro* [38]. The tolerogenic NIMA effect was further confirmed *in vivo* in an adoptive transfer experiments. Donor T cell expansion decreased significantly more in mice receiving NIMA--exposed T cells than those receiving control T cells. When adoptively transferred into third-party B6C3F1 (H-$2^{b/k}$) mice, donor T cell expansion did not decrease, thus indicating the antigen specificity of the NIMA effects. As a consequence of reduced allogeneic T cell responses, bone marrow transplantation from an NIMA-exposed donor significantly decreased the mortality and morbidity of acute GVHD.

Donor immunity in allogeneic HSCT also harnesses beneficial graft-versus-leukemia (GVL) effects and, therefore, allogeneic HSCT represents a very potent form of immune therapy against hematological malignancies [80, 81]. We also found that NIMA-mismatched HSCT favors T cell immune reconstitution and permits GVL effects, thus resulting in a marked improvement in outcome after HSCT. GVL activity is strongly correlated with the severity of GVHD [20, 24]. Optimal GVL response occurs when alloantigens are expressed on both APCs and tumor tissues, and cytotoxic T lymphocyte (CTL) responses are crucial for GVL [53]. In these experiments, the use of an NIMA-exposed donor did not completely inhibit the activation of alloreactive T cells and the development of GVHD. This may thus permit the induction of the GVL effect, which is primarily mediated by both donor $CD4^+$ and $CD8^+$ T cells in this model [67].

We then tested whether IPA exposure influences the outcome after allogeneic HSCT [38]. A female B6 mouse was mated with a male B6D2F1 mouse and exposed to IPA-H-2^{d} from her H-$2^{b/d}$ offspring during pregnancy. T cells isolated from IPA-exposed mice were not hyporesponsive to IPAs, and bone marrow transplantation from an IPA-exposed donor did not result in a clinically significant reduction of acute GVHD compared with transplantation from a control donor. Our results closely correlate with the clinical observation that the incidence of severe GVHD was significantly lower in HSCT from a NIMA-mismatched donor than from an IPA-mismatched donor [27].

Why does NIMA-exposure drive the immune system toward tolerance more potently than maternal exposure to IPAs? There are several explanations for the differential immunogenecity. First, NIMAs are exposed to the fetus perinatally, when the immune system is immature. Second, NIMAs can also be exposed to a child after birth via breast-feeding in addition to *in utero* exposure. Third, a mother might become sensitized to paternal minor histocompatibility antigens (mHAs) during pregnancy [74, 76]. Indeed, multiparity promotes priming to male-specific mHAs [29].

Breast-milk is rich in maternal MHC antigens in both cellular and soluble forms [3, 12, 41]. Foster nursing experiments in murine models of skin and heart transplantation showed the requirements of both pregnancy and breast-feeding to achieve long-term allograft survival [3, 87]. Exposure to NIMA both *in utero* and by breast-feeding appears to generate higher levels of maternal microchimerism than *in utero* exposure alone, and the degree of microchimerism, which correlates with a prolonged survival in maternal heart grafts [3]. T cells recognize alloantigens via direct or indirect allorecognition pathways. Since breast-milk contributes to the establishment of microchimerism in the offspring [3, 12, 41], an indirect allorecognition pathway may be involved in the induction of tolerogenic NIMA effects. To address this issue, pregnant female mice were injected with an MHC allopeptide [3]. This procedure induced the long-term tolerization of offspring T cells toward the corresponding antigens, thus suggesting that alloantigen exposure to the fetus can affect developing immune systems via an indirect allorecognition pathway. Interestingly, Ilan et al. [28].suggested that oral tolerance could be induced in adulthood by feeding recipient mice with proteins extracted from recipient's splenocytes for 11 days after allogeneic HSCT.

Central and/or peripheral tolerance mechanisms may contribute to the tolerogenic NIMA effects. The injection of allogeneic spleen cells to neonates eliminates donor reactive T cells and prolongs skin graft survival [17]. In these mice, intrathymic deletion of donor reactive T cells was evident in association with intrathymic donor microchimerism; however, intrathymic microchimerism is transient and thus donor reactive T cells can be generated later in the thymus. In a patient who is tolerant to a maternal kidney allograft, donor--specific CTL activity was hardly detected, but restimulation with donor stimulator cells and interleukin (IL)-2 restored this activity, thus indicating that anti-donor CTL precursors were not completely deleted [10]. We recently found that $CD4^+$ $CD25^+$ regulatory T cells were primarily responsible for the tolerogenic NIMA effects, since depletion of these cells from the donor inocula resulted in abrogation of the NIMA effects [38]. These results suggest that NIMA-reactive T cells presumably escape thymic negative selection induced by intrathymic microchimerism, and long-lasting stable tolerance requires peripheral tolerance mechanisms.

Although antigen-nonspecific suppressive effects of

$CD4^+CD25^+$ regulatory T cells have been well recognized, our results suggested the tolerogenic NIMA effect to be antigen specific. Antigen-specific expansion of regulatory T cells requires a T cell-deficient environment and stimulation with the corresponding antigens in the presence of IL-2 [32, 46, 70]. Therefore, the lymphopenic status after myeloablative conditioning and allogeneic HSCT in the presence of NIMAs may thus allow an expansion of NIMA-specific $CD4^+CD25^+$ regulatory T cells [16, 86]. Recently, Sanchez-Fueyo et al. [55] demonstrated that the function of these regulatory T cells is dependent on specific T cell receptor stimulation, providing evidence for their specificity in the regulation of alloimmune responses. Currently, calcineurin inhibitors such as cyclosporine and tacrolimus are standard use for GVHD prevention in allogeneic HSCT. These agents interfere with the regulatory T cell function through an IL-2-dependent mechanism and thereby may suppress the NIMA effects. The use of immunosuppressants that do not interfere with these regulatory T cells, such as sirolimus and mycophenolate mofetil, therefore enable us to achieve an optimal NIMA effect [8, 31].

CONCLUSION

The tolerogenic NIMA effect is a form of naturally acquired immunological hyporesponsiveness, which is thought to be a sequel of fetal or neonatal tolerance to NIMAs. Our findings highlight the essential role of $CD4^+CD25^+$ regulatory T cells in the induction of NIMA effects after allogeneic HSCT, thus supporting the hypothesis that NIMA-specific tolerance is maintained by an immunological mechanism which is actively induced following exposure to NIMAs.

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