Long-term feto-maternal microchimerism revisited

Microchimerism and tolerance in hematopoietic stem cell transplantation

Tatsuo Ichinohe

Department of Hematology and Oncology; Graduate School of Medicine; Kyoto University; Kyoto, Japan

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Abbreviations: DTH, delayed-type hypersensitivity; GVHD, graft-versus-host disease; HSC, hematopoietic stem cell; IPA, inherited paternal antigen; mHAg, minor histocompatibility antigen; NIMA, noninherited maternal antigen; PBMC, peripheral blood mononuclear cell; TGF, transforming growth factor; T_E cells, T effector cells; T_R cells, T regulatory cells

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Correspondence to: Tatsuo Ichinohe;
Email: nohe@kuhp.kyoto-u.ac.jp

Bidirectional fetal-maternal cell traffic during pregnancy gives rise to stable persistence of minute amounts of allogeneic cells both in the mother and in her offspring, a phenomenon called long-term fetal or maternal microchimerism. Over the past decade, increasing attention has been devoted to elucidating the biological relevance of such reciprocal microchimerism, unveiling its conflicting roles in either immune sensitization or tolerance induction against fetal or maternal alloantigens. Recent studies in mice and humans have highlighted the significance of fetal-maternal microchimerism in the induction and maintenance of CD4⁺CD25⁺ and CD8⁺ T regulatory cells that counterbalance the immune responses to fetal or maternal antigens mediated by T effector cells. Consistent with these observations, T-cell-replete hematopoietic stem cell transplantation between mutually microchimeric mothers and their HLA-haploidentical offspring has been shown to be feasible, although the degree of microchimerism-associated tolerance appears to substantially differ among the cases. Since *in vitro* or *trans-vivo* assays to detect antigen-specific tolerance in the context of the T regulator versus T effector balance are now available, future clinical studies incorporating these tests into the criteria for donor selection are warranted to more precisely define the relevance of fetal-maternal microchimerism in allo-tolerance and immune homeostasis after hematopoietic stem cell transplantation.

Introduction

Long-lasting bidirectional tolerance between the donor and recipient without excessive pharmacologic immunosuppression has been one of the ultimate but unfeasible goals in allogeneic hematopoietic stem cell (HSC) transplantation. Especially in the setting of HSC transplants from genetically HLA-disparate donors, substantial proportions of patients receiving intensive immunosuppressive agents still suffer from graft rejection or severe graft-versus-host disease (GVHD), either of which significantly compromises post-transplant survival outcomes. Given the ever-increasing number of HSC transplants with the use of donors other than HLA-identical siblings, it is crucially important to further elucidate the immunologic mechanisms by which HLA-incompatible HSC allografts are successfully accepted by some recipients but not by others.

Fetal-maternal microchimerism is a form of naturally acquired microchimerism that commonly occurs among eutherian mammals through two-way nucleated cell exchanges between the mother and fetus during the course of normal pregnancy.¹⁻³ With the help of flow cytometric cell sorting and highly sensitive polymerase chain reactions, minute amounts of hematopoietic cells and/or DNA of fetal origin can be detected in the blood and tissues of the respective mothers as long as decades postpartum,^{4,5} while low levels of maternal cells can be identified not only in the various tissues of the fetus but in the peripheral circulation of the

immunocompetent adult offspring.^{6,7} Thus far the biological relevance of such long-term persistent microchimerism is not fully characterized, although earlier observations suggested its possible roles in immune sensitization against fetal or maternal alloantigens.¹⁻³ Long-term fetal microchimerism in parous women has been reported to be associated with susceptibility to a variety of autoimmune disorders and protection from certain cancers.^{1,2,8,9} Similarly, persistence of maternal microchimerism in immunocompetent offspring was suggested to be involved in the pathogenic mechanisms underlying immune-mediated pediatric diseases such as neonatal lupus syndrome and biliary atresia.^{10,11}

In addition to functioning as sources for alloantigens to prime immune effectors, fetal-maternal microchimerism is now emerging as a crucial player involved in the induction and maintenance of pregnancy-associated immune tolerance toward fetal inherited paternal antigens (IPAs) in mothers as well as toward noninherited maternal antigens (NIMAs) in their progeny.¹² In the past several years, the feasibility of HSC transplants from HLA-haploidentical microchimeric donors has been explored to improve donor availability and transplant outcomes based on the hypothesis that maternal donors with fetal microchimerism are rendered immunologically hyporesponsive to their offspring and vice versa, offspring donors harboring maternal microchimerism are rendered tolerant to both their mothers and HLA-haploidentical siblings expressing NIMAs as mismatched histocompatibility antigens. In this brief article, we will discuss the promises and challenges of such HSC transplants from donors with fetal or maternal microchimerism in the light of future directions to take more advantage of microchimerism-associated immune equilibrium by employing assays to evaluate and means to enhance the robustness of IPA- or NIMA-specific tolerance probably determined by balance between T regulator and T effector cells responsive to fetal or maternal alloantigens.

Clinical and Experimental Observations Linking Long-Term Fetal-Maternal Microchimerism to Hematopoietic Cell Allograft Tolerance

A possible linkage between fetal microchimerism and HSC allograft tolerance was initially suggested by Tokita et al. who reported a case of a woman with refractory thymic carcinoma successfully treated with an infusion of allogeneic peripheral blood stem cells from her HLA-haploidentical daughter.¹³ This woman was revealed to harbor microchimerism presumed to have originated from the daughter prior to and 11 months after the stem cell infusion. It was speculated that the infused donor cells could survive in face of the maternal immune system probably because the recipient mother had already become rendered hyporesponsive to IPAs of her daughter. Furthermore, the mother had no clinical manifestations of GVHD throughout the treatment period, implying the presence of reciprocal tolerance to NIMAs in the daughter. Soon thereafter, Ochiai et al. reported a case of a young male patient with refractory leukemia who underwent successful non-T-cell-depleted peripheral blood stem cell transplantation from his microchimeric mother, setting the first milestone for the clinical development of HSC donor selection based on fetal-maternal microchimerism.¹⁴ Notably, it was recently reported that HLA-haploidentical lymphocyte infusions from mothers harboring long-term fetal microchimerism were also effective in eradicating Epstein-Barr virus-positive T-cell lymphoproliferative diseases developed in their offspring without causing obvious GVHD.¹⁵

A close association of maternal microchimerism with acquisition and maintenance of specific tolerance to NIMAs has been more clearly demonstrated through a series of studies in mice and humans.¹⁶⁻¹⁸ With the use of the murine F₁ x P back-cross breeding model (B6 x BDF1) to generate H-2^{b/b} offspring mice exposed to H-2^d as NIMA in utero and/or via breastfeeding, Zhang and Miller for the first time demonstrated that NIMA-exposed mice showed prolonged acceptance of maternal skin allografts while the period

of graft survival was positively correlated with the amount of naturally transferred maternal T cells in their lymph nodes.¹⁶ Andrassy and Kusaka et al. employed the same murine model and showed that NIMA-exposed offspring achieving tolerance to fully allogeneic H-2^{d/d} heart allografts had relatively higher levels of maternal microchimerism in the lymphoid organs.¹⁷ Importantly, human fetal lymphoid organs also contain microchimeric maternal cells,^{7,18} and subsequent experiments in mice and humans revealed that the tolerogenic NIMA effects were dependent on CD4⁺CD25⁺ T cells capable of suppressing alloreactive T-cell responses to maternal antigens.^{18,19} These observations strongly suggested the crucial role of microchimeric maternal cells to generate NIMA-specific CD4⁺CD25^{high}Foxp3⁺ T regulatory cells in the offspring. It is also an intriguing and important question whether a similar mechanism can operate in parous women harboring fetal microchimerism because CD4⁺CD25⁺ T regulatory cells were also demonstrated to be involved in the induction and maintenance of maternal tolerance to the fetus in a murine model.²⁰

Clinical Results of HLA-haploidentical Hematopoietic Stem Cell Transplantation from Donors Harboring Fetal or Maternal Microchimerism

In support of the above-mentioned observations, it has been reported that HSC transplants from mothers or NIMA-mismatched siblings confer superior outcomes as compared to those from fathers or siblings without NIMA mismatch as HSC donors,²¹⁻²³ probably suggesting better posttransplant immune reconstitution in the presence of IPA or NIMA mismatch. To further extend the availability of HLA-incompatible donors with the help of remote influence of fetal-maternal tolerance, we have explored the feasibility of T-cell-replete HSC transplantation from HLA-haploidentical family donors harboring persistent fetal or maternal microchimerism in the peripheral circulation.²⁴ These transplants involved three different types of donor-recipient combination: (i) transplantation from mothers

Figure 1. Cumulative incidence of acute GVHD in patients who underwent hematopoietic cell transplantation from an HLA-haploidentical related donor harboring fetal or maternal microchimerism. Cumulative incidence of moderate-to-severe (grade 2–4) (A) and severe (grade 3 or 4) (B) acute GVHD according to the type of HLA mismatch among 34 evaluable patients who received T-cell-replete HSC transplantation from an HLA-haploidentical related donor harboring fetal or maternal microchimerism. Solid line, transplants from mothers to their offspring (IPA mismatch in the GVH vector); dashed line, transplants from offspring to their mothers or to NIMA-mismatched siblings (NIMA mismatch in the GVH vector). This research was originally published in *Blood*.²⁴ Ichinohe T, et al. Feasibility of HLA-haploidentical hematopoietic stem cell transplantation between noninherited maternal antigen (NIMA)-mismatched family members linked with long-term fetal-maternal microchimerism. *Blood* 2004; 104:3821–8. © The American Society of Hematology.

carrying microchimerism of fetal origin to their respective offspring, a setting that microchimerism-associated allotolerance in the donor might favorably modulate GVH responses against IPAs; (ii) transplantation from offspring carrying maternal microchimerism to their mothers or

(iii) to their NIMA-mismatched HLA-haploidentical siblings, a setting that microchimerism-associated allotolerance in the donor might ameliorate GVH responses against NIMAs.³

Based on this scheme, we analyzed clinical outcomes of 35 patients with

high-risk hematologic malignancy who received T-cell-replete HSC allografts from donors with fetal or maternal microchimerism.²⁴ With the use of conventional tacrolimus-based GVHD prophylaxis, all patients achieved durable engraftment and 19 (56%) of 34 evaluable patients developed grade 2–4 acute GVHD (Fig. 1A). In this study, the presence of NIMA mismatch in the GVH direction was associated with lower risk of developing severe acute GVHD as compared with IPA mismatch (Fig. 1B), although this observation should be reevaluated in a larger prospective cohort. Recently, we have updated information regarding survival of these patients and confirmed that such transplants conferred acceptable probabilities of long-term disease-free survival (Fig. 2). A series of case reports have also shown the feasibility of HLA-mismatched HSC donor selection based on fetal-maternal microchimerism,¹² but some cases are still associated with graft rejection and severe acute GVHD.²⁵ Furthermore, longer follow-up of these patients lead to a paradoxical observation that substantial proportions of survivors could discontinue administration of immunosuppressive agents despite the frequent occurrence of chronic GVHD requiring systemic treatment,²⁶ raising a new question as to the role of fetal-maternal microchimerism as an indicator of clinically relevant tolerance toward IPAs or NIMAs.

Equilibrium between T Regulatory Cells and T Effector Cells as a Mechanism to Determine the Net Immune Responses against Fetal or Maternal Alloantigens

Apparently conflicting results with respect to HSC transplants from microchimeric donors can be clearly reconciled by recent studies demonstrating that the tolerance associated with long-term fetal-maternal microchimerism would be determined by the balance between T regulatory cells (T_R) and T effector (T_E) cells specific for IPAs or NIMAs.^{27–29} Cai et al. explored the role of minor histocompatibility antigen (mHAgs) HA-1 in the allograft tolerance of three patients who received kidney transplants from an HLA-matched but HA-1-mismatched family member.²⁷ By

Figure 2. Long-term survival of patients who underwent hematopoietic cell transplantation from an HLA-haploidentical related donor harboring fetal or maternal microchimerism. Kaplan-Meier estimates of overall survival with a median follow-up of 4.6 years in 35 patients who received T-cell-replete HSC transplantation from an HLA-haploidentical related donor harboring fetal or maternal microchimerism for advanced hematologic malignancies. Solid line, transplants in remission; dashed line, transplants in chemotherapy-refractory disease.

analyzing peripheral blood mononuclear cells (PBMCs) obtained from these recipients with the use of HA-1-specific tetramer staining, they were able to detect two distinct tetramer-positive CD8⁺ T-cell populations: the tetramer brightly positive population with characteristics of T_E cells that produces IFN γ and the tetramer dimly positive population with characteristics of T_R cells that produces IL-10 and transforming growth factor (TGF) β . Trans-vivo injection of PBMCs of these three patients into the footpads of severe combined immunodeficiency mice lead to IL-10 and/or TGF β -regulated delayed-type hypersensitivity (DTH) responses to HA-1 peptide. The suppression of these T_E cell functions by HA-1 tetramer dimly positive CD8⁺ T_R cells was dependent on IL-10, TGF β and cytotoxic T lymphocyte-associated antigen 4 (CTLA-4). Intriguingly, these recipients were proven to harbor microchimerism of HA-1-positive dendritic cells. This report was the first demonstration of coexistence of microchimeric cells carrying mHAg, T_E cells responding to mHAg and T_R cells that are capable of suppressing mHAg-specific T_E cell functions, thus suggesting an essential role of microchimeric cells as a reservoir of alloantigens that are required for maintenance of both mHAg-specific T_E and T_R cells.

Recently, van Halteren et al. provided more robust evidence that parous women and their healthy offspring can persistently harbor mHAg-specific CD8⁺ T_R cells bearing low-avidity T-cell receptors as well as CD8⁺ cytotoxic T cells reactive to the same mHAg. With the use of PBMCs of healthy mother-offspring pairs mismatched for HY and/or HA-1 antigens, the presence of mHAg-specific CD8⁺ T_R cells was analyzed in the trans-vivo DTH assays and a substantial proportion of mothers and offspring were found to harbor mHAg tetramer-dimly positive T_R cells functioning in a CTLA-4-dependent manner. Importantly, they also showed that the functional balance between T_R cells versus T_E cells significantly differ among individuals, providing a clue to explain the differential immunogenicity against IPAs or NIMAs observed in the setting of clinical HSC transplantation.

In a subsequent study using a murine model, Dutta et al. showed that the levels and tissue distribution of maternal microchimerism are strongly correlated with maintenance of NIMA-specific T_R cells in mice developmentally and neonatally exposed to NIMA.²⁹ Levels of maternal microchimerism were positively correlated with NIMA-specific suppression of both DTH and in vivo mixed

lymphoproliferative reactions. Remarkably, when mice were exposed to NIMA only in utero and lacked oral exposure to NIMA via breastfeeding, maternal microchimerism was lost in association with emergence of sensitized DTH responses against NIMA. Collectively, these findings support the scenario that modes of immune responses toward IPAs in parous females as well as those toward NIMAs in offspring are determined by the positive or negative net balance between IPA- or NIMA-specific T_R cells and T_E cells, which might be correlated with levels of persistent fetal or maternal microchimerism and specific donor-recipient combinations of histocompatibility antigens.³⁰

Future Directions and Conclusions

Accumulating lines of evidence from mice and humans have indicated the close association between long-term fetal-maternal microchimerism and the development of IPA- or NIMA-specific CD4⁺CD25⁺ and CD8⁺ T_R cells. Since the immune equilibrium in favor of T_R cells versus T_E cells appears to be necessary for the maintenance of IPA- or NIMA-specific tolerance, future studies to improve the outcome of HSC transplants should aim at identifying means to enhance such tolerance in HSC donors harboring long-term fetal or maternal microchimerism. Because breastfeeding appears to be an indispensable mechanism to establish robust NIMA-specific tolerance, it is intriguing to investigate whether oral administration of NIMA peptide can promote the generation of NIMA-specific T_R cells in adult offspring previously exposed to NIMAs. In a similar fashion, whether oral or non-oral exposure to IPAs may affect IPA-specific immune responses in mothers harboring fetal microchimerism remains an open question. Since laboratory tests to evaluate T_R versus T_E balance such as allopeptide-specific tetramer staining or trans-vivo DTH assays have now become available, further clinical studies incorporating these tests into the criteria for microchimeric HSC donor selection are warranted to clarify the relevance of fetal-maternal microchimerism in establishing allospecific tolerance after hematopoietic stem cell transplantation.

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