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Microchimerism: tolerance vs. sensitization

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Abstract

Purpose of review—The bidirectional exchange of cells, both mature and progenitor types, at the maternal–fetal interface is a common feature of mammalian reproduction. The presence of semiallogeneic cells in a host can have significant immunological effects on transplantation tolerance and rejection. Here, we review recent advances in this area.

Recent findings—Maternal microchimerism (MMc) in blood and various organs was found to be directly correlated with noninherited maternal antigen (NIMA)-specific CD4⁺ regulatory T cells (Tregs), in F₁ backcross mice. In humans, MMc induced NIMA-specific FoxP3⁺ CD4 Tregs in lymph nodes and spleen of fetuses. Tolerance to NIMA⁺ allografts could be predicted in mice by measuring levels of the NIMA-specific Tregs in offspring before transplantation. On the contrary, fetal microchimerism (FMc) in multiparous female mice was largely confined to CD34⁺ hematopoietic stem cells (HSCs) and was associated with sensitization rather than Treg induction. The recent discovery of a ‘layered’ T-cell development in humans whereby fetal HSCs are more likely to produce Tregs than adult HSCs, which may explain why MMc often induces tolerance, whereas FMc tends to induce sensitization.

Summary—Microchimerism may cause tolerance resulting in acceptance of an allograft bearing antigens shared by the microchimeric cells. However, microchimerism may also cause sensitization resulting in rejection. Distinguishing these effects prior to the transplant may revolutionize the field of living-related renal transplantation wherein MMc and FMc can exert a powerful influence on graft outcome.

Keywords

microchimerism; noninherited maternal antigens; T regulatory cells; tolerance

Introduction

Exchange of cells in fetal and neonatal life results in microchimerism in mother and offspring, which has life-long immunological influence in hosts.

Biology of placenta

The biology of the placenta is key to understand the mechanism of fetal–maternal cell exchange. The types of placenta in mammals are very divergent. One of the main sources of heterogeneity is in degree of penetration of the uterus by trophoblasts. In epitheliochorial

placentation (cattle, goats, pigs, horses), there is no invasion of the uterus, whereas in hemochorial placentation (human, monkeys, mice, rats) invasion is very severe [1]. In hemochorial placentation, trophoblasts invade the endothelial cell lining of maternal blood vessels resulting in presence of maternal blood in intervillous space. Therefore, fetal tissues are bathed in maternal blood resulting in an easy access of maternal cells to the fetus and vice versa.

Exchange of cells at feto–maternal interface

Exchange of cells through the placenta is bidirectional [2]. Fetal cells can cross the placenta and seed in different maternal tissues, which results in fetal microchimerism (FMc) [3,4]. Similarly, maternal cells can also cross placenta and result in maternal microchimerism (MMc). MMc has been detected in different organs of offspring including blood [5], spleen [6], brain [7], heart [6], lungs [6], liver [8], and pancreas [9].

Exchange of progenitors in pregnancy

Microchimerism has been demonstrated in a wide variety of cell types in peripheral blood and lymphoid organs including T cells [10•,11,12], B cells [11], dendritic cells [10•,13], and macrophages [10•]. Stevens *et al.* [14] demonstrated presence of maternal hepatocytes in liver, renal tubular cells in kidney, and β cells in pancreas in infants. Bianchi *et al.* [3] reported presence of fetal cells in maternal blood many years after pregnancy. These findings indicate transfer of multipotent/pluripotent stem cells during pregnancy, which continuously divide and replenish the pool of microchimeric cells [15]. The earliest findings of presence of fetal stem cells in mother are from Khosrotehrani *et al.* [16] and O'Donoghue *et al.* [17]. Fetus-derived mesenchymal stem cells were detected in the bone marrow of all women decades after pregnancy with male fetuses [17]. The mesenchymal stem cells were able to differentiate into chondrocytes, osteocytes, and adipocytes. We also found high levels of hematopoietic progenitor cells of offspring origin in the bone marrow of B6 female mice after four to five pregnancies [18]. In another study, Khosrotehrani *et al.* [11] crossed Rag-deficient female mice, which lack T and B cells, with wild-type male mice and recovered functional T and B cells of fetal origin from the spleen of the female mice during pregnancy. Bianchi *et al.* [3], by a nested PCR detected CD34⁺ male progenitor cells in the peripheral blood of women who were pregnant with male offspring many years before the blood sampling.

Evidence of transfer of maternally derived progenitor cells into fetuses was reported by Chen *et al.* [19] who injected human mesenchymal stem cells into pregnant rats and showed that the MSCs crossed the placenta and seeded into different organs, in which they differentiated into tissue-specific parenchymal cells. The human MSCs expressed integrins and vascular endothelium growth factor receptor 1 (VEGF-R1). It helped them to migrate across the placenta, as there was also a gradient of VEGF from the maternal blood to fetal blood. The presence of progenitor cells of maternal origin in adult offspring was reported by our lab [20•]. Lineage-negative c-kit⁺ maternally derived stem cells were present in both bone marrow and hearts of the offspring. Presence of the maternally derived cardiac stem cells correlated with presence of cardiomyocytes of maternal origin indicating a local pool of maternally derived progenitor cells as a source of MMc in the heart. However, in this study we did not attempt to distinguish cardiac myocytes from Hoechst 'side population' cardiac progenitor cells [21•], so we cannot exclude the possibility of MMc in this separate cardiac progenitor pool. Mesenchymal stem cells were also identified in cultured bone marrow cells collected from the offspring. All neonates and 80% of the adult offspring tested by a qPCR assay had acquired maternally derived bone marrow HSC. However, only 50% of them had detectable levels of maternally derived differentiated cells, indicating

either failure to engraft fully, or active elimination of the differentiated cells by the adaptive immune system of the host. The most compelling evidence for the latter mechanism comes not from MMc in F₁ backcross offspring (BDF₁ females × B6 males), in which noninherited maternal antigen (NIMA)-specific sensitization is rare [22] but from FMc in the control (B6 × females BDF₁ males) female B6 breeders. These multiparous females, the majority of which (five out of eight) exhibited cellular sensitization to H-2^d antigen in delayed type hypersensitivity (DTH) cell transfer assay, all harbored high levels of FMc in their bone marrow HSC.

Nursing as a source of maternal microchimerism

In addition to transfer of cells across placenta in fetal life, maternal cells can be obtained through nursing in neonatal life. When H2^b B6 females × BDF₁ males ('NIPA') control offspring were foster nursed by BDF₁ mothers, two out of seven offspring exhibited H2D^d microchimerism in their livers [20•], consistent with the finding by Zhou *et al.* [8], who showed that green fluorescent protein (GFP⁺) maternal cells present in the breast milk mainly localized in livers of neonates after nursing. However, how maternal cells manage to escape gastric acidity and get into blood circulation of neonates is not clear.

Microchimerism and tolerance

The proposal that microchimerism induces tolerance was first put forward by Starzl *et al.* [23]. Since then, the functional linkage between microchimerism and tolerance has been difficult to establish [24,25]. Burlingham *et al.* [24] reported a causative link between cytotoxic T lymphocyte (CTL) unresponsiveness and donor microchimerism in a patient tolerant to a kidney allograft from his mother. The patient's peripheral blood mononuclear cells (PBMCs), which contained approximately one donor cell in 10⁴–10⁵ cells by PCR and Southern blot assay, had very little donor specific CTL activity in primary mixed lymphocyte culture, but his anti-NIMA CTL response recovered in secondary culture with interleukin 2. When the rare donor cells present in recipient PBMCs were positively selected and added to the secondary culture, the added NIMA⁺ fraction markedly inhibited CTL response to the maternal donor; however, no inhibition of maternal anti-third party CTL activity was found [24]. This finding indicated that donor microchimerism was responsible for the reduced antidonor CTL activity in 1^o culture; however, we did not rule out an effect of host cells bearing acquired donor antigen, a phenomenon called trogocytosis [26]. Cells acquiring allogeneic antigens by trogocytosis or endocytosis can also become regulatory [27]. We have found that even though spleen and lymph node of the NIMA^d-exposed mice had very low levels of MMc (0.01–0.001%), more than 1% of the antigen-presenting cells (APCs) in these organs had acquired maternal H-2^d class I and class II antigen on the cell surface [10••] indicating the potential of 'semi-direct' [28] pathway of maternal antigen presentation to host T cells. If the APC in the aforementioned human study also had acquired maternal human leukocyte antigen (HLA)-B antigen, they would likely have been present in the donor cell-enriched fraction of PBMCs, and may have caused the observed CTL inhibition [24]. Though trogocytosis is a random process, several biological significances of trogocytosis have been described [29]. For example, clearance of CD8⁺ T cells occurred by antigen-specific cytolysis when they acquired cognate major histocompatibility complex (MHC) class I and peptide ligand. CD4⁺ T cells acquiring MHC class II and costimulatory molecules might act as antigen presenting cells [30]. Interestingly natural killer cells [27], CD4⁺, and CD8⁺ T cells [31] acquiring HLA-G by trogocytosis can also acquire regulatory functions.

The hypothesis that microchimerism is a cause of tolerance was supported by an elegant study in which B6 mice were engrafted with H8 cells that constitutively expressed H2D^b-

restricted GP33 epitope of lymphocytic choriomeningitis virus (LCMV) [25]. GP33-specific CTLs were unresponsive after LCMV challenge of H8-microchimeric B6 mice; experimental depletion of the microchimeric cells restored the activity of GP33-specific T cells.

Maternal microchimerism and transplantation tolerance in animal models

A mouse F_1 backcross breeding model ($B6 \times BDF_1$) originally described by Zhang and Miller [32] is the most commonly used mouse model to study effects of NIMAs. When $B6$ ($H2^{b/b}$) male mice are mated with BDF_1 ($H2^{b/d}$) female mice, half the offspring inherit $H-2^d$ from the mother, whereas the other half is $NIMA^d$ -exposed $H2^b$ -homozygous offspring. The breeding pair is switched (BDF_1 male $\times$ $B6$ female control mating) to obtain $NIPA^d$ (non-inherited paternal antigen) control mice (also $H2^{b/b}$) that have the same genetic background as $NIMA^d$ -exposed mice; however, they are not exposed to the $H-2^d$ [or $DBA/2$ (Dirty Brown Agouti/2) minor H] antigens. Andrassy *et al.* [33] performed heterotopic heart transplantation and showed that about 50% of the male $NIMA^d$ -exposed offspring accepted heart allografts bearing NIMA, whereas the nonexposed control offspring uniformly rejected the allografts by day 11 after transplant. The surviving heart allografts in the $NIMA^d$ -exposed group were devoid of any vascular intimal hyperplasia, a common sign of chronic rejection, indicating that the tolerance induced by the NIMA exposure is not only resistant to acute rejection but also to chronic rejection. The $NIMA^d$ tolerance effect, although present [34], was less pronounced in female offspring (25% $DBA/2$ heart allograft survival) suggesting a possible hormonal effect limiting tolerance in females [35].

Tolerance to bone marrow transplantation has also been detected using the $BDF_1 \times B6$ breeding model [36]. In this study, irradiated BDF_1 mice were transplanted with bone marrow cells from $B6$ and either the $NIMA^d$ -exposed or $NIPA^d$ control mice. The group receiving bone marrow cells from $NIMA^d$ -exposed mice exhibited less graft-versus-host disease (GVHD) than the other two groups. Additionally, the NIMA effect was abrogated when $CD4^+CD25^+$ Treg-depleted NIMA-exposed donor cells were transferred, indicating that the Tregs were indeed involved in NIMA-mediated GVHD prevention [36]. Molitor-Dart *et al.* [37] showed that the splenocytes of $NIMA^d$ -exposed mice could regulate DTH swelling induced by tetanus toxoid recall response in presence of BDF_1 antigens, but the splenocytes of $NIPA^d$ control mice did not. The bystander DTH suppression was found in 50% of the $NIMA^d$ -exposed offspring and was dependent on IL10 and tumor growth factor (TGF)- β , indicating a role of Treg cells. When carboxyfluorescein succinimidyl ester (CFSE)-labeled splenocytes from the $NIMA^d$ -exposed mice were injected into a naïve BDF_1 , host $CD4^+$ T cells proliferated less and expressed higher levels of TGF- β on anergic $CD4^+$ T cells than the splenic $CD4^+$ T cells control mice. The $NIMA^d$ -exposed mice that became tolerant to NIMA-expressing $DBA/2$ heart allografts had IL-10 and TGF- β -producing $CD4^+CD25^+$ T cells in the regional lymph nodes and allograft infiltrates. A recent study [22] reported NIMA-tolerance in a transgenic mouse model. In this model, homozygous $H-2^K$ -offspring exposed to maternal $H-2K^b$ (MHC class I) had TcR transgenic $CD8^+$ T cells specific for K^b antigen. Though the offspring did not delete K^b -specific $CD8^+$ T cells in thymus, they were nonetheless tolerant to heart allografts expressing the NIMA. The NIMA tolerance was abrogated by depleting $CD4^+$ T but not $CD8^+$ T cells.

A study by Mold *et al.* [38] (reviewed in [39••]) has had a major impact on the field, shifting the focus of NIMA effects in humans to maternal antigen-specific $FoxP3^+CD4^+$ T regulatory cells. This study showed: (a) the presence of microchimeric maternal cells in different organs including human 18–20 week fetal lymph nodes, spleen, liver, heart, and lungs; and (b) suppressed proliferation of fetal T lymphocytes in response to maternal APCs but not to unrelated third party APC. The suppression of proliferation was mediated by

maternal antigen-specific CD4⁺ CD25⁺ Tregs, which were generated upon proliferation of fetal T cells in response to maternal alloantigens in fetal lymph nodes. The acquisition of FoxP3 and suppressive function could be abrogated by blocking TGF- β signaling.

It should be noted that other types of T cells, apart from FoxP3⁺ CD4 Tregs may mediate regulatory effects of microchimerism-exposure in humans. For example, van Halteren *et al.* [40] have recently described two-way interactions between mothers and sons, resulting in induction of minor H antigen (miHA)-specific CD8, FoxP3⁻, T regulatory cells. These CD8 T cells tended to be HLA-tetramer^{dim}, CTLA-4⁺, and suppressed DTH reactions. Although detected in some mothers toward the HY miHA of the son, mother's CD8 T cells contained more HLA-tetramer^{bright} CD8 CTL and were biased toward effector, rather than regulatory functions.

A study performed in our lab showed that MMc was correlated with reduced proliferation of, and increased cell-surface latent TGF- β expression on, CD4 T⁺ cells from NIMA^d-exposed offspring exposed to maternal antigens *in vivo* [10••]. MMc was also directly correlated with % suppression of tetanus toxoid-induced recall DTH response in the presence of maternal antigens. These findings indicated a causative link between MMc and NIMA-specific Tregs, which are required for NIMA-specific transplant tolerance. By measuring pretransplant activity of NIMA-specific Tregs with DTH assay using splenocytes collected after hemisplenectomy, we successfully predicted allograft outcomes in NIMA^d-exposed offspring [41••]. All (12/12) of NIMA^d-exposed offspring that did not suppress tetanus toxoid-induced recall DTH swelling (nonregulators) rejected NIMA-containing allografts by day 15, whereas five out of six DTH 'regulator' NIMA^d-exposed offspring accepted the allografts (more than 100 days). Allograft rejection in the DTH- 'nonregulator' offspring could be prevented by adoptive transfer of CD4⁺ CD25⁺ Tregs but not CD4⁺CD25⁻ or CD8 T cells from the tolerant (more than 100 days.) regulator offspring. Additionally, we found the NIMA-tolerant and regulator offspring had higher levels of GFP⁺ MMc than the NIMA-rejector and nonregulator offspring (Dutta *et al.*, manuscript in preparation).

Mechanisms of microchimerism-induced tolerance

Though microchimerism and tolerance have been linked, it is not clear how microchimerism induces tolerance. Bemelman *et al.* [42] found that below a certain threshold of 'macrochimerism', low numbers of donor HSCs fail to cause clonal deletion. Instead, the resulting microchimerism induced 'infectious' tolerance, which was mediated by Tregs. In the case of maternal antigen-specific Tregs nursing is important, suggesting a role for oral tolerance. In absence of nursing, the offspring rejected maternal antigen-containing allografts [32] and became sensitized to maternal antigens [10••], all of which was accompanied by loss of detectable MMc. Aoyama *et al.* [43••] reported that the NIMA effect in allogeneic bone marrow transplantation could be potentiated when the offspring were nursed. Oral exposure to soluble MHC antigens present in breast milk [44] can generate TGF- β -producing antigen-specific CD4 Tregs [45,46].

MMc in CD11b- and CD11c-positive cells sorted from spleen and bone marrow has been detected [10••]. These cells express maternal antigens in context of MHC class II and may perform direct antigen presentation to offspring Tregs. The maternal antigens can also be processed and presented by offspring APCs (indirect presentation). This suggests the possibility for inducing a NIMA-specific Treg able to recognize maternal antigens via both direct and indirect presentations. Tregs having both indirect and direct allospecificity have been shown to be especially potent in inducing tolerance to allografts [47].

Another mechanism of induction of tolerance by microchimerism may be clonal deletion. Maternal APCs present in the fetal thymus and bone marrow can cause deletion of the NIMA-specific lymphocytes. GFP⁺ maternal thymic epithelial cells have been detected in the NIMA^d-exposed offspring [10••]. In principle, these cells can present antigens during negative selection of T cells in the thymus. However, Akiyama *et al.* [22] failed to detect deletion of CD8⁺ Treg cells exposed to their NIMA ligand. The situation may be different for B cells. Vernochet *et al.* [48] described partial deletion of B cells having high affinity for the NIMA; however, B cells having low affinity for the NIMA were not clonally deleted.

Summary

Microchimerism induces not only tolerance but also sensitization. What determines the outcomes of microchimerism is not clear. It has been reported that several factors, such as presence or absence of oral exposure to antigens, and extent of microchimerism may be critical factors.

Sensitization vs. tolerance

Although there is increasing evidence indicating a tolerizing effect of microchimerism in human and experimental sensitization limiting the extent of microchimerism is also possible [49]. Most Rh⁻ women born of an Rh⁺ mother failed to produce antibody against the Rh antigen when they gave birth to Rh⁺ children, indicating that exposure to the maternal Rh antigen had resulted in tolerance to the antigen in the Rh⁻ women. However, the few Rh⁻ women born of Rh⁺ mothers that did make anti-Rh antibody had much worse Rh disease in their babies than Rh⁻ birthed by Rh⁻ mothers. We found that 12/12 NIMA^d-exposed 'nonregulator' offspring that did not have detectable Treg activity rejected NIMA-containing allografts by d15 posttransplants [41••]. One of these 12 mice also had NIMA-specific effector T cells detected by a DTH assay. This mouse rejected the graft by d7 posttransplant rather than 9–10 days, indicating sensitization to the NIMA. Why MMC causes tolerance vs. sensitization is not clear.

Nursing and oral tolerance

In absence of nursing, offspring were sensitized to maternal antigen in adult life and lost MMC indicating an active elimination of maternal cells in absence of Tregs [10••]. This indicates in absence of oral exposure to maternal antigens maternal antigen-specific Tregs are not generated, which is necessary to suppress maternal antigen-specific effector T cells.

Split tolerance and 'split' chimerism

The type of microchimerism is also important in the outcome. In an elegant study, Chan *et al.* [50] made a mixed bone marrow chimera in nonobese diabetic mice in which chimerism was only in T-cell lineage (unilineage microchimerism). Though the mice were tolerant to allogeneic T cells they rejected allogeneic skin and islet grafts, a phenomenon called 'split tolerance'. In contrast, B6 chimeric mice had multilineage chimerism and accepted the allografts. Consistent with the idea of 'split tolerance', we also found that five out of eight B6 female mice crossed with BDF₁ male mice were sensitized to fetal H2^d antigens and had FMc only in lineage-negative c-kit⁺ cells in very high levels [18]. On the contrary, three out of eight mothers that had FMc in different organs including heart, lungs, and liver were not sensitized to the fetal antigens. Moreover, two of them had fetal antigen-specific Tregs. These findings indicate that not only quantity but also quality of microchimerism matters in the outcome.

Immunity

In addition to mediating sensitization and transplantation rejection, it has been suggested that FMc is involved in autoimmune diseases. The involvement of FMc in systemic sclerosis has been demonstrated by different studies [6,51,52]. Women with systemic sclerosis had higher level of male DNA in their blood than healthy controls [53]. Some women with systemic sclerosis who gave birth to male children decades ago had male DNA at very high levels.

Conclusion

It is clear that outcome of MMc is mostly tolerance, whereas FMc induces mostly sensitization. Offspring are exposed to maternal antigens/cells in fetal and neonatal life when their immune systems are immature, which may result in lifelong tolerance to allograft containing the alloantigen [54]. However, mothers are exposed to fetal antigens/cells when their immune systems are mature, resulting mainly in sensitization to fetal antigens. In addition to this, oral exposure to maternal antigens in offspring generates maternal antigen-specific Tregs (discussed above), which mediates tolerance. A very recent study by Mold *et al.* [55••] looked into molecular and functional differences between fetal and adult hematopoietic stem and progenitor cells (HSPCs). When they stimulated naïve CD4⁺ T cells with allogeneic PBMCs, fetal naïve T cells were more efficient to produce Tregs than adult naïve T cells. Fetal CD4⁺ T cells also expressed different gene signatures than their adult counterpart. To investigate whether fetal and adult CD4⁺ T cells are derived from two different pools of HSPCs, they injected HSPCs isolated from adult bone marrow, fetal bone marrow, and fetal liver into humanized severe complex immune deficiency (SCID) mice and looked for cell differentiation. Fetal bone marrow- and liver-derived HSPCs produced significantly higher levels of Tregs than adult bone marrow-derived HSPCs. Therefore, fetal T cells are biased to immune tolerance, which may partly explain why exposure to maternal antigens in fetal life mostly induces immune tolerance.

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References and recommended reading

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- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 444-445).

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Key points

- Maternal microchimerism (MMc) is directly linked with noninherited maternal antigen (NIMA) specific tolerance.
- NIMA-induced tolerance could be predicted before transplantation by measuring activity of NIMA-specific Tregs.
- Stem cells of maternal origin present in adult off-spring may replenish the MMc pool.
- Microchimerism may induce not only tolerance but also sensitization.
- Fetal microchimerism only in the c-kit⁺ pluripotent progenitor bone marrow subpopulation was found in mothers sensitized to offspring antigens, whereas multilineage microchimerism was present in tolerant mothers. This situation suggests a mechanism for maintaining sensitization vs. tolerance in multiparous females.