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Immunobiology of exposure to non-inherited maternal antigens

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TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Evidence of the NIMA effect in human transplantation
3.1. Limitations of the NIMA effect
4. Development of animal models of NIMA effect
4.1. Effects of NIMA on T cell responses
4.2. NIMA effects on B cells
4.3. Mouse strain differences in the NIMA effect
5. Maternal Microchimerism
6. Immunocompetence of the fetal/neonatal immune system
7. Maternal microchimerism and autoimmunity
8. Possible mechanisms behind the NIMA tolerance effect
8.1. Clonal deletion and anergy
8.2. ACAID-like induction of NIMA tolerance
8.3. Oral tolerance and the NIMA effect
9. Conclusions
10. References

1. ABSTRACT

Exposure to non-inherited maternal antigens (NIMA) has life-long immunological consequences that may result in tolerance or immunity to these antigens. Gaining understanding of the mechanisms behind these NIMA effects will impact many areas of immunology. This review summarizes new discoveries relevant to autoimmunity and organ transplantation regarding exposure to maternal antigens. In light of these studies, as well as unpublished data from our lab, we conclude that the effect of neonatal exposure to maternal antigens has profound impact on clinical and experimental transplantation and autoimmunity, and important implications for the immune system development.

2. INTRODUCTION

Despite many important advances in clinical transplantation, the lifelong reliance on immunosuppressive medication has severe long-term consequences in terms of increased risk of infection and invasive cancer, as well as renal failure from nephrotoxic effects of calcineurin inhibitors. Chronic rejection also remains one of the leading causes of graft failure in renal transplants. Although it is known that human leukocyte antigen (HLA) matching does improve graft acceptance, it is not always practical and in any case cannot provide the whole answer to the problem of poor long-term graft survival. The ultimate solution to the rejection problem lies in the induction and maintenance of tolerance, tolerance being defined as lifelong survival of a well functioning graft without the dependency on immunosuppressive therapy.

In the search for natural mechanisms of allotolerance, Owen *et al* (1) discovered almost fifty years ago that Rh-negative mothers of Rh^+ babies had a significantly reduced likelihood of forming anti-Rh antibodies if their own mothers had been Rh^+. Three decades later, Claas *et al* (2) repeated this observation in the HLA system while analyzing anti-HLA antibodies in multiply transfused, highly sensitized patients awaiting renal transplant, when they discovered that such individuals frequently failed to make antibody against the non-inherited maternal antigens (NIMA) of their mothers. No such "immune privilege" was afforded to HLA antigens not inherited from the father.

A possible explanation for this apparent B-cell hyporesponsiveness to maternal antigens was that during early fetal and neonatal life, maternal lymphocytes and/or soluble antigens enter the offspring's circulation; rather than activating a humoral response, the maternal cells/antigens may instead induce immune tolerance in the baby. Although the fetal and maternal blood circulations are separated, numerous studies have demonstrated the passage of cells across the fetal-maternal interface in either direction (3). It has also been found that leukocytes (4) and soluble HLA antigens (5) are present in breast milk and are therefore transferred to the nursing neonates. This exposure to maternal antigens has a lifelong influence on the immune system. The most compelling evidence of the influence of exposure to maternal antigens on the immune system has been found in studies evaluating the role of NIMAs in transplantation. This review will focus on

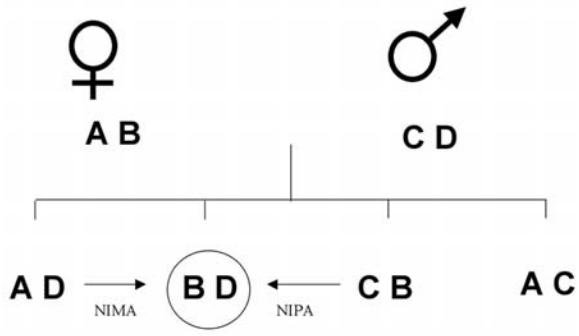

Figure 1. Immunogentics of NIMA and NIPA. The offspring that is circled has the following familial antigens. Inherited maternal antigen (IMA) = B, Inherited paternal antigen (IPA) = D, Non-inherited maternal antigen (NIMA) = A, Non-inherited paternal antigen (NIPA) = C, Arrows indicate a hypothetical NIMA or NIPA transplant.

describing these studies of NIMA in clinical and experimental transplantation models, along with several factors influencing, and possible mechanisms underlying, the NIMA phenomenon.

3. EVIDENCE OF THE NIMA EFFECT IN HUMAN TRANSPLANTATION:

To begin to discuss the role of neonatal tolerance to maternal antigens in organ transplantation, a nomenclature of the familial antigens is needed. Figure 1 illustrates the immunogenetics of non-inherited and inherited major histocompatibility complex (MHC) antigens in a hypothetical family. If the mother has alleles A and B of the gene for an HLA antigen and the father has alleles C and D of the same gene, each child will receive one allele from each parent. If the circled child in Figure 1 inherited the B allele from mother, than the A allele is the NIMA. Similarly, this child inherited the D allele from father, making the C allele the non-inherited paternal antigen, or NIPA. Although this child did not inherit the NIMA-A, they were presumably exposed to this antigen *in utero* and possible again orally via nursing. It is thought that this exposure to the NIMA has a life-long influence on the host immune response.

To determine whether this exposure was of clinical benefit to patients who later received an allograft as an adult, Burlingham *et al* (6) analyzed the outcome of a primary renal transplantation between siblings. They found that transplant recipients of a one-HLA haplotype-mismatched allograft that expressed the NIMA HLA experienced significantly better long term graft survival than did recipients of a NIPA-mismatched graft. The sibling-donated, NIMA-mismatched allograft recipients fared as well as HLA-identical sibling graft recipients at ten years post transplantation. Furthermore, a NIMA effect was found in patients receiving a kidney transplant from an unrelated cadaveric donor, but only for the HLA-A antigens (7). In this study, recipients from donors mismatched for an HLA-A that was identical to the NIMA HLA-A antigen of the recipient's mother, fared better than did recipients of HLA-A fully matched grafts. Taken together these data suggest that the NIMA effect is caused by an active process of regulation of the immune response in the offspring.

In support of the findings in solid organ transplant, more evidence for a NIMA effect was found in two independent studies of patients receiving a haploidentical parental or sibling hematopoietic stem cell (HSC) transplant. van Rood *et al* (8) and Ichinohe *et al* (9) found a powerful tolerogenic effect on NIMA exposure suppressing graft-versus-host disease (GVHD) in bone marrow transplantation between living related donors. The lowest incidence of acute GVHD was seen in HSC transplantation between offspring→mother (maternal NIMA-mismatched) and sibling→sibling (sibling NIMA-mismatched). Offspring→father and NIPA-mismatched sibling HSC transplants has a significantly higher incidence of GVHD. Most recently, Japanese transplant centers have successfully used NIMA-mismatched sibling and maternal donors in stem cell transplantation introducing the parameter of mutual feto-maternal microchimerism in donor and recipient in the selection of donors. Specifically, Kodera *et al* (10) studied transplantations from one-HLA haplotype-matched family donors who showed pre-transplant microchimerism of non-inherited maternal antigens. HSC transplants were performed without T-cell depletion but with tacrolimus prophylaxis. The rates of engraftment and survival in this circumstance were similar to those obtained with transplantation from HLA-matched sibling donors.

The excellent kidney graft survival ad relatively low rates of GVHD after bone marrow or HSC transplantation in HLA-identical donor-recipient pairs remains the "gold standard" of comparison for evaluating the impact of NIMA-HLA; i.e. to say that NIMA siblings do as well as HLA-identical siblings is to say they do as well as the very best transplants. However, it should be noted that for minor H antigens, the very same rules and nomenclature outlined in Figure 1 for HLA alleles also apply. Since it is likely that mother will be heterozygous for one or more minor H antigens, one may assume that the HLA-identical sibling transplant is heavily influenced by NIMA effects on the offspring's response to sibling NIMA-minor H antigens. Because of this, HLA-identical sibling transplants may be a fertile ground for future analysis of the mechanism of the NIMA effect, as suggested elsewhere (11).

3.1 Limitations of the NIMA Effect

Taken together, these observations strongly support the existence of a NIMA effect that has a significant beneficial impact on the outcome of allogeneic transplantation. However, several important limitations remain. First, maternal kidney grafts do not fare better than paternal grafts (12). The weakness of the NIMA effect when mother is the donor implies that other variables can impact the realization of the NIMA effect in the allograft recipient. One of these factors is the immune status of the maternal donor, which is very different than that of the sibling donor. Not only have pregnant women been shown

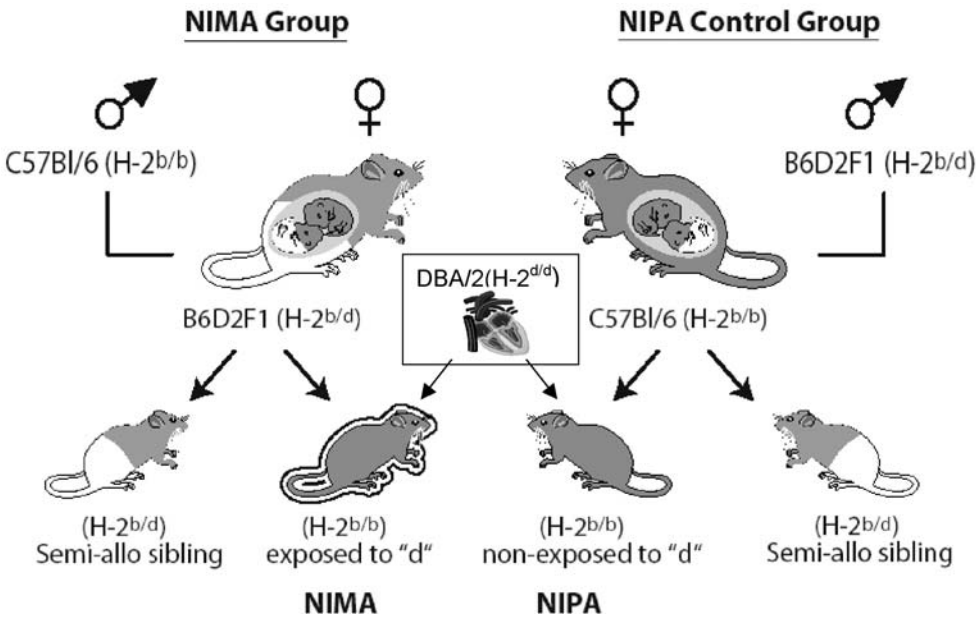

Figure 2. NIMA Mouse Model Breeding Scheme. Unlike the human outbred situation, only 2 familial MHC haplotypes are present, rather than 4. Reproduced with permission from (19), Journal of Immunology copyright 2003.

to form antibodies against the paternal HLA (13), but also the maternal transplanted passenger leukocytes present in the graft will be confronted with the inherited paternal antigens (IPA) of the recipient and be re-stimulated. Since the mother has been exposed to the mismatched IPA of the offspring during adult life when her immune system is fully mature, memory T and B cells residing in the transplanted organ may have a greater chance of mounting a humoral and cellular response against the IPA (14, 15). In the case of a NIMA-mismatched sibling transplant, donor leukocytes will have been exposed to inherited maternal antigens (IMA) on the recipients cells [footnote] during fetal and neonatal life. The "graft versus host" reaction to the IPA in the case of a maternal donor is likely to be vigorous and sustained whereas that of the NIMA sibling donor to the IMA will be maternally influenced, and hence, the early rejection will be more limited. Thus, the NIMA effect in sibling transplantation is really a "double NIMA" effect, i.e. bi-directional (host versus donor, donor versus host), whereas the NIMA effect in the case of a maternal graft is unidirectional (host versus donor only). The unidirectional NIMA effect is clearly weaker. When mother- and father-donated kidney transplants are compared in a donor-specific transfusion (DST) protocol, which reinforces the NIMA tolerance effect in vitro (16), a pronounced trend toward improved maternal graft survival was seen (17).

Second, in the sibling renal transplantations, there was a highly significant increase in early acute rejection episodes in the NIMA-mismatched recipients as compared to the NIPA-mismatched controls (6). Subsequent re-analysis of the data of Burlingham *et al* (6) revealed that the early-acute rejection phenomenon is confined to HLA-DR mismatched donor-recipient pairs, suggesting a specific sensitization to class II antigens of the mother (Burlingham, WJ and Heisey, DM, unpublished). This suggested that maternal cells may actually prime the offspring's T effector cells *in utero*, an idea that has recently been proven experimentally for maternal minor H (NIMA) antigens using human cord blood T lymphocytes and HLA-tetramer analysis (18). In keeping with the idea of a "double NIMA" effect in siblings the pro-inflammatory conditions of transplant surgery and ischemia-reperfusion injury may re-activate the allo-specific memory T effector cells of both host and donor (passenger leukocytes) origin. The resulting early acute rejection episode may be less damaging to the graft than a late acute rejection episode, since the latter would occur when the patient is being less closely monitored and thus not promptly diagnosed and treated. It is also possible that the rejection responses to NIMA are more highly regulated than those to paternal and third party alloantigens. This would be the case if maternal cells and soluble antigens have induced not only T effector cells, but also a parallel set of T regulatory cells that migrate from the blood and lymph nodes to the graft to dampen the acute rejection response (this possibility will be addressed in detail, below). Thus, a primed rejection response to NIMA may occur, but these are readily reversed by drug therapy. Rejection is rarely seen after the first six months in NIMA siblings, whereas late (>6 mo.) rejection is common in NIPA sibling transplants (6).

4. DEVELOPMENT OF ANIMAL MODELS OF NIMA EFFECT

In order to address the mechanism of neonatally-induced tolerance to maternal antigens, animal models are needed. Andrassy *et al* (19) were able to reproduce the essential features of the NIMA effect seen in clinical kidney transplantation using a heterotopic heart transplant model with F1 backcross mice as recipients. Figure 2

depicts the breeding scheme of this mouse NIMA model. C57BL/6 male (B6 H-$2^{b/b}$) mice are crossed with a (B6 x DBA/2)F1 (H-$2^{b/d}$) female, resulting in either H-$2^{b/b}$ or H-$2^{b/d}$ offspring. The H-$2^{b/b}$ offspring (outlined in white, Figure 2) is the one of interest since it did not inherit the H-2^{d} from mother, but was intimately exposed to this antigen *in utero* and again orally via nursing. This NIMA breeding scheme is the same as the one originally described by Zhang and Miller (20). To control for background genes resulting from the F1 backcross, we switched the parental halpotypes so that the female was the B6 and the male was the BDF1. The resulting H-$2^{b/b}$ offspring (the NIPA control) will have the same distribution of non-MHC background genes as the NIMA^{d} exposed mouse, but will not have had the neonatal exposure to the H-2^{d} haplotype. Animals were given a fully allogeneic DBA/2 (H-$2^{d/d}$) heart heterotopic heart transplant and monitored for graft survival. It was found that 57% of DBA/2 heart allografts were accepted (graft survival >180 days) without any drug or conditioning treatment in the NIMA^{d}-exposed F1 backcross H-$2^{b/b}$ mice, whereas the NIPA^{d} (non-exposed) H-$2^{b/b}$ controls uniformly rejected around day 11 post transplantation. Furthermore, the long term surviving allografts in the NIMA^{d}-exposed mice lacked vascular intimal hyperplasia that is normally associated with chronic rejection indicating true tolerance to the NIMA^{d} expressing allografts rather than simply the exchange of acute for chronic rejection (19).

Interestingly, the NIMA^{d} exposed mice experienced what appeared to be acute rejection episodes as indicated by a weakening of the heartbeat early (2-3 days) post-transplant. This is very similar to the kidney transplant data where NIMA mismatched kidney transplant recipients also show an increase in acute rejection episodes, but then go on to recover and enjoy long term allograft acceptance (6). Another interesting aspect of the NIMA effect in mice was that in female mice only 25% long-term graft survival was observed. While this was significantly different from female NIPA controls ($p<.05$) (Molitor, Andrassy, unpublished), it was less than the 55% tolerance rate seen in male NIMA offspring. This may be related to the dependence of cardiac allograft rejection on CD4^{+} T cells (21, 22), and the estrogen-dependent enhancement of the female CD4^{+} T cell response to foreign antigens (23).

4.1. Effects of NIMA on T Cell Response

In the Andrassy study (19), we reported that NIMA^{d} exposed offspring produced less IFN-gamma, IL-2, and IL-5 by ELISpot as compared to the B6 control when stimulated with DBA/2 or B6D2F1 stimulator cells pre-transplant. However, since this publication, we have found that when one compares the NIMA^{d} exposed mice to the proper NIPA^{d} control mice, one no longer sees a difference in the production of IFN-gamma (Molitor M et al, manuscript in preparation). However, when the post-transplant production of IFN-gamma and IL-10 were compared, NIMA^{d} exposed tolerant mice had a significant decrease in the production of IFN-gamma and increase in IL-10 production compared to NIPA^{d} non-exposed controls (Molitor et al, manuscript in preparation). In addition to the increase in IL-10 production, NIMA^{d} exposed tolerant mice had significantly more cell surface TGF-beta positive cells not only in their spleen and lymph nodes, but also in the heart graft itself, compared to NIPA^{d} controls post transplant. These results indicate the potential induction of an IL-10 and/or a TGF-beta producing T regulatory cell by an organ transplant that "recalls" a neonatal exposure to NIMA. These NIMA^{d} tolerant mice are able to regulate a DTH response to BDF_1 antigen post transplant, a response that can be uncovered with the addition of anti-IL-10 or anti-TGF-beta antibodies, or by depleting the CD4^{+}CD25^{+} T cells, further supporting this idea of the development of a NIMA specific T regulatory cell (Molitor et al, manuscript in preparation).

As noted above, weak NIMA effects may be enhanced by donor-(NIMA+) specific transfusion (16). Preliminary studies of NIMA^{d} exposed mice pre-transplant that have been treated with a DST of B6D2F1 splenocytes show an increase their cell surface latent TGF-beta^{+} T cells compared to DST-treated NIPA^{d} controls. Furthermore, these T regs are able to regulate a DTH to the NIMA^{d} antigen, further supporting the idea of a NIMA^{d} specific T regulatory cell that may be re-activated by antigen exposure in the adult (Molitor et al, manuscript in preparation).

Further evidence for induction of NIMA-specific CD4^{+} T reg cells comes from the work of Akiyama *et al* (24). They studied the effect of NIMA exposure in mouse heart transplants using a double-transgenic model involving CBK transgenic mice, i.e. CBA [H-2^{k}] mice expressing K^{b} MHC class I transgene. In this model, BM3.3 (CBA anti-K^{b}) CD8^{+} T cell receptor (TCR) transgenic male mice were crossed with (CBA x CBK)F1 (K^{b+}) females heterozygous for the K^{b} transgene. K^{b} negative offspring were given a CBK (K^{b+}) transgenic heterotopic heart transplants and monitored for graft survival. Controls included the IMA offspring (K^{b+}), and the offspring of a BM3.3 male and CBA female cross (non-exposed to K^{b}). All non-exposed control mice uniformly rejected their CBK grafts within 18 days post-transplant and exhibited a potent pro-inflammatory response to K^{b+} donor cells. IMA offspring that inherited the K^{b} gene and deleted the BM3.3 Tg TCR^{+} T cells uniformly accepted a CBK heart graft. However, the NIMA mice displayed long-term survival of their allografts (MST = 60 days) and displayed a reduced Th1 type response and an increase in Th2 type cytokines when stimulated with K^{b+} cells (24). It is important to note that unlike IMA control siblings, the transgenic CD8^{+} T cells specific for the K^{b+} cells were not deleted in the NIMA exposed mice, ruling out deletion as the possible mechanism of NIMA tolerance in this model (24). The presence of CD4^{+} T cells secreting type 2 cytokines suggests a possible role for immune deviation. However the involvement of CD4^{+} regulatory T cells in this model was not investigated. Indeed, the results of this study, along with others suggesting intact adult CD8^{+} CTL responses to NIMA (25), may help account for early NIMA-associated acute rejection mediated in part by CD8^{+} primed T memory cells to allo-MHC class I, while the inhibition of Th-1 CD4^{+} T cell response may account for prolonged graft survival after the NIMA-specific early rejection crisis.

4.2. NIMA Effects on B Cells

The influence of NIMA exposure on the humoral immune response in adults was the first definitive evidence for a NIMA effect in humans (1, 2). While helper T cell defects may partly account for these results, Vernochet *et al* (26) asked whether NIMA had a direct influence on the development of fetal and neonatal B lymphocytes. Using the 3-83 B cell transgenic mouse model, in which B cells recognize the MHC class I molecules H-2K^k and H-2K^b, with a high or moderate affinity, respectively, they analyzed the fate of transgenic B cells that had been exposed to maternal H-2K^k or maternal H-2K^b. The results were compared to those seen in non-exposed controls. The authors of this study found that maternal cells influence the development of fetal and neonatal B cells. Specifically when the offspring's B cells have a high affinity for the NIMA, a partial deletion of B cells occurred, and a modulation of the antigen receptor on remaining high affinity B cells in late gestational fetuses was observed. Furthermore, the remaining high affinity splenic B cells specific for the NIMA showed an impaired proliferative ability, down-regulated sIgM, and showed increased expression of active caspase-3, suggestive of apoptosis induced by B cell receptor cross-linking. These results suggest that fetal high affinity B cells exposed to NIMA display a state of tolerance. In contrast, when the NIMA is recognized by a transgenic B cell with lower affinity to NIMA, the authors observed a very different situation of B cell activation which included increased B cell numbers in bone marrow and spleen, increased proliferation of B cells, up-regulation of sIgM, expression of CD69, and invasion of T cell follicular zone by B cells in the spleen (26). The results from this study indicate that B cell receptor affinity for the NIMA plays a major role in the timing and quality of the response elicited by the interaction.

Effects of NIMA on B cell responses were also observed in follow-up studies to the paper by Andrassy *et al* (19). Analysis of the NIMA^d exposed and NIPA^d control mice post-transplant for production of H-2^d specific antibodies revealed no differences in the production of IgG_1 antibodies. However, NIMA^d heart allograft-tolerant mice produced significantly less complement fixing H-2^d specific IgG_2 antibodies compared to NIPA^d controls (Molitor et al, manuscript in preparation).This indicates that the B cells in the NIMA^d transplanted mice are not class switching efficiently from the production of non-complement fixing to complement fixing antibodies. Perhaps this is one reason why we did not see chronic rejection in the NIMA^d exposed transplanted mice (19), since chronic rejection is thought to be in part antibody mediated (27).

4.3. Strain Differences in the NIMA Effect

Claas *et al* (2) found only 50% of highly sensitized patients failed to make antibody to their particular NIMA. The NIMA effect is also preferentially seen in particular mouse strain combinations. By using the same backcross breeding strategy employed to create the NIMA^d model (figure 2), we were able to create an additional three NIMA models: NIMA^b (C3H male x B6C3F1 female), NIMA^k (B6 male x B6C3F1 female), and the NIMA^{d2} (C3H male x C3D2F1 female). The NIMA^{d2} offspring, when challenged with a DBA/2 heart, displayed a significant degree of tolerance compared with the NIPA^{d2} controls. However, the NIMA^k and NIMA^b models did not show a NIMA tolerance effect when challenged with a C3H or B6 transplant, respectively. In fact, the NIMA^b mice produced significantly more NIMA-specific complement fixing IgG_2 antibody than their NIPA^b controls and the NIMA^k mice produced more IFN-gamma than their NIPA^k controls when stimulated with maternal antigen (Molitor, ML and Haynes LD, manuscript in preparation). These data suggest that exposure to NIMA does not always induce a tolerizing effect, but that several other factors may contribute to the process of sensitization versus tolerization.

5. MATERNAL MICROCHIMERISM

The fact that the NIMA effect is observed in adults indicates a powerful lifelong influence of maternal antigen exposure on the host immune system. A possible explanation for the longevity of the NIMA effect is that maternal cells are commonly transferred into the fetus during pregnancy in both humans and mice. In fact, maternal microchimerism has been found to persist from fetal life into adulthood in at least half of all humans tested (28, 29). Similarly, there have been several studies reporting the presence of maternal microchimerism in mouse models that demonstrate the frequent occurrence in fetal, neonatal, and adult lymphoid and hemtopoietic tissues of maternal cells that have been transferred *in utero* or through breastfeeding (4, 20, 30). This maternal microchimerism has been associated with autoimmunity (31, 32) and allograft tolerance (33).

More recently, Kaplan and Land (34), using a neomycin resistance gene (neoR) as a marker for maternal cells, have shown that normal mice possess small numbers of maternal cells in spleen, thymus and brain. Remarkably, neoR$^+$ cells were found in lymphoid and non-lymphoid tissues (including the brain) of virtually all neoR$^-$ neonatal and adult offspring born to neoR+ mothers. Interestingly, there was a tendency for higher levels of maternal microchimerism to be found in MHC homozygous mice (34). Vernochet *et al* (26), using an enhanced GFP-transgenic mother, found that maternal cells frequently gain access to the fetus not only in syngeneic pregnancies, but also in allogeneic and outbred mouse crosses. These maternal cells included B, T and NK cells, and were found to persist into adulthood primarily in the bone marrow and thymus (26).

Interestingly in the study by Vernochet *et al* (26), they found that lymphoid organs (including thymus, spleen, and liver) of adult offspring harbor maternal cells more frequently than do the same fetal organs, suggesting that maternal cells can engraft and proliferate in the adult. The finding of maternal cells in non-lymphoid tissues such as the brain (34) also suggests that migrant maternal cells may possess unexpected plasticity (35, 36). Taking this idea of maternal microchimerism one step further, Stevens *et al* (37) asked whether maternal stem cells can not only cross the placental barrier, but also differentiate into tissue-

specific phenotypes in the fetus. Using a technique that allowed for identification of multiple phenotypic markers that could be detected concurrently with the FISH technique in the same cells, a small number of maternal cells expressing sarcomeric alpha-actin, a specific marker for cardiac myocytes, were found in the hearts of infants. These results indicated that maternal microchimerism can occur in parenchymal tissues as well as hematopoietic tissues in the neonate. Maternal cells permanently engrafted into the tissues of the offspring could act as constant low level NIMA-antigen source perpetuating the tolerance mechanism, either by shedding antigens or by host antigen presenting cells (APC) antigen acquisition (38, 39).

Maternal microchimerism may also occur through breast-feeding of the offspring. Using a GFP transgenic mouse model to monitor maternal cell traffic to offspring, GFP^+ cells were not only found in the immune system of GFP^- fetuses grown in GFP (+/-) mothers during normal pregnancy, but GFP^+ cells were also detected in GFP^- neonates born to GFP^- mothers that had been fed by GFP^+ foster nursing mothers (4). These maternal milk leukocytes were mainly found in the livers of neonates.

6. IMMUNOCOMPETENCE OF THE FETAL/NEONATAL IMMUNE SYSTEM

As we have seen, there is now abundant evidence that maternal cells and antigens expressing NIMA enter into the fetus during pregnancy and into the baby during nursing. The immunocompetence of the developing immune system to respond to these invasions by mother is a key issue in understanding the mechanism of the NIMA effect. Fetal and neonatal immune responses have been considered to be immature and weaker than that of adults (40). Conflicting results have been reported on the capacity of cord blood cells to mediate alloreactive cytolytic activity, with some authors describing a normal function (41), and others describing an absent or reduced response (42). An important contribution was made by was made by Ridge *et al* (43) who showed that fetal/neonatal T cells were fully immunocompetent to respond to mature adult DC; such priming of neonatal T cells resulted in immunity, rather than tolerance in the adult. In related studies of immunity to the HY minor antigen in mice, Anderson and Matzinger (44) reported that donor microchimerism arising from skin-graft passenger leukocytes could induce either immunity or tolerance, depending on the state of the host T cells: a mature immune T cell repertoire was primed by microchimerism for skin graft rejection, while newly arising T cells in the chimeric thymus were tolerized by the same microchimerism (44). By these criteria, maternal cells are likely to have both tolerizing and immunizing effects on the developing immune system of the offspring. The balance of these maternal effects may determine whether tolerance or autoimmunity results from the maternal-fetal interaction.

7. MATERNAL MICROCHIMERISM AND AUTOIMMUNITY

The persistence of maternal cells in offspring implies that the offspring is tolerant to a low level of maternal antigen, and the existence of a beneficial NIMA effect in transplantation implies that such "low-zone" tolerance can be amplified to achieve tolerance to a substantial antigen load such as a heart or kidney transplant. However, maternal microchimerism has also been associated with many autoimmune diseases indicating that tolerance to NIMA may be broken. For example, maternal microchimerism was found to be significantly more frequent in patients with systemic sclerosis (SSc) versus controls (72% vs 22%), although the actual levels of maternal microchimerism did not differ significantly in patients with SSc versus controls who were found to be positive (45). Similarly, when peripheral blood and muscle biopsies from children with dermatomyositis were examined for maternal microchimerism, the frequency of maternal microchimerism was significantly increased in the blood and muscles biopsied from diseased children then from unrelated controls and unaffected siblings (46). Maternal microchimerism has also been found in higher proportions in children with juvenile idiopathic inflammatory myopathies compared to unaffected controls (47). Lastly, maternal cells have been found in the atrioventricular node and in the myocardium in infants with neonatal lupus syndrome (up to 2% in cases versus 0.1% in controls of all cells in some sections). Up to 80% of these maternal cells expressed tissue specific markers suggesting that this tissue-specific maternal microchimerism could become the target for an autoimmune response (37). Although, it has been alternatively hypothesized that maternal cells may migrate to areas of tissue damage secondarily and function beneficially in repair (37, 48).

While the studies mentioned above support the idea that maternal microchimerism may contribute to autoimmune diseases, many studies have shown that maternal microchimerism is common among healthy individuals. Because of this, other environmental or genetic factors must be involved. Certainly specific HLA alleles, especially DRB and DQA1, have been associated with autoimmune diseases (49). These associations of certain HLA specificities or alleles with autoimmune diseases has been regarded as evidence that autoreactive T lymphocytes should be MHC-restricted and recognize particular self-determinants. Sharing of autoimmune-prone MHC alleles by mother and baby may contribute to the breaking of self-tolerance (Stevens A, manuscript in press). Yet, Zanelli et al (50), through studying rheumatoid arthritis in humans and animal models, has proposed an alternative hypothesis which is that HLA does not predispose one to autoimmune diseases, but rather fails to provide efficient protection due to impaired T-cell mediated immune regulation.

8. POSSIBLE MECHANSIMS BEHIND THE NIMA TOLERANCE EFFECT

While there is substantial evidence of a beneficial NIMA effect in clinical and experimental transplantation, little is known about the mechanism whereby exposure to maternal cells and soluble HLA influence the offspring's alloimmune response. Several possible mechanisms have

been proposed, among them clonal deletion, anergy, and the production of NIMA-specific T regulatory cells.

8.1. Clonal Deletion and Anergy:

It has been hypothesized that fetal/neonatal T and B cells responding to maternal microchimerism present in the thymus and bone marrow during development may become clonally deleted or anergized. The study by Vernochet *et al* (26) suggests that clonal deletion and anergy are indeed outcomes of high affinity interactions of B cells with maternal MHC/NIMA as evidence by partial deletion of these high affinity B cells and decreased proliferative capabilities and defective class switching of the high affinity B cells remaining. On the other hand, neither low affinity B cells/NIMA interactions (26) or high affinity $CD8^+$ T cell NIMA interactions (24) led to clonal deletion or anergy. In the case of $CD8^+$ T cells specific for minor H antigens, it has recently been found that low avidity TCR-antigen interactions are a feature of T reg cells, while CTL/effectors have high avidity (11). Furthermore, Mommas et al (18), established the presence of circulating NIMA-minor H specific T cells in the cord blood which bound tetramers with either low or high avidity. In vitro culture enriched for high avidity CTL responses against NIMA-expressing cells. This substantiates the priming effect of NIMA while the presence of low-avidity, tetramer dim $CD8^+$ T cells indicates a possible source of NIMA-specific T reg cells. Perhaps it is the balance of these two populations that results in either tolerance or immunity to NIMA.

8.2. ACAID-like Induction of NIMA Tolerance

The eye, like the pregnant uterus, is one of several specialized organs/tissues that displays immune privilege in that it permits foreign tissue grafts to enjoy prolonged or indefinite survival (51, 52). Immune privilege in the eye is an active process and has been termed anterior chamber-associated immune deviation (ACAID) (51, 52). ACAID occurs when antigenic maternal is captured by distinctive local APCs that have been bathed in the aqueous humor containing immune modulators such as TGF-beta and travels through the blood into the spleen. In the marginal zone of the spleen, the antigen presenting cells, including $CD1d^+$ marginal zone B cells, stimulate $CD4^+$ NK T cells necessary for the induction of $CD4^+$ and $CD8^+$, TGF-beta producing T reg cells. The result is a systemic immune response that inhibits $CD4^+$ T effector cells that mediate delayed-type hypersensitivity (DTH), and T helper cells that help B cells to secrete compliment-fixing antibodies. Antigen specific T cells differentiate into regulatory cells that persist in the peripheral circulation enforcing tolerance (51-53).

The feto-placental unit within the pregnant uterus is thought of as an immunological privileged site and it has been hypothesized that the NIMA effect could arise through mechanisms like that of ACAID (54). Amniotic fluid is thought to be like the aqueous humor of the eye in that it contains a large amount of TGF-beta, an immune modulator known to play a central role in ACAID (55). Evidence supporting this idea comes from a study in which APCs exposed *in vitro* to aqueous humor from the eye or amniotic fluid acquired the capacity to induce ACAID *in vivo* when pulsed with antigen and injected intravenously into naïve recipients (55, 56). Therefore, it could be that the NIMA effect is induced in the child as a result of drinking amniotic fluid containing NIMA bearing maternal cells and soluble antigens, together with TGF-beta and other potential immune modulating factors, thereby modulating the APCs in the Peyer's patches inducing T regulatory cells (54).

8.3. Oral Tolerance and the NIMA Effect

The critical role of breast milk in the NIMA effect was demonstrated in the Andrassy *et al* (19) $NIMA^d$ mouse study where both *in utero* and oral exposure to the $NIMA^d$ alloantigens were necessary for this tolerogenic effect. Many other studies have shown the importance of oral exposure to maternal antigen via nursing in transplantation beginning with Campbell et al (57) who found that maternal renal transplant recipients who had been breast fed experienced better long term graft survival compared with non-breast fed patients.

Oral administration of antigen is known to lead to a systemic, antigen-specific, state of immunological hyporesponsiveness, and is thought to play a large role in the induction of tolerance to NIMA. Feeding low does of antigen has been found to induce cell populations that actively suppress bystander T cell proliferation indicating the induction of regulatory cells (58), and feeding low doses of antigen multiple times or continuously improves the suppressive tolerance (59). More specifically, oral administration of antigen has been shown to expand $CD4^+CD25^+$ T cells which are able to suppress a DTH reaction further supporting the role for the induction of antigen specific T regulatory cells (60). Furthermore, orally tolerized T cells display an inability to clonally expand, enter the B cell follicles, and support B cell responses following mucosal challenge (61).

Breastfeeding can be considered to be a low dose, continuous feeding regimen of NIMA HLA. Molitor *et al* (5) recently quantified the amount of soluble maternal antigen in breast milk and cord plasma to determine the extent of neonatal exposure to NIMA. ELISA analysis of cord blood from three genetically HLA-A2 negative babies born to $HLA\text{-}A2^+$ mothers, and from two HLA-A3 negative babies born to $HLA\text{-}A3^+$ mothers, revealed that a significant amount of soluble NIMA HLA-A was detected in the cord blood. The level of NIMA-A2 or –A3 in the cord blood was approximately 10% of the predicted value for a baby genetically positive for that allele (5). Furthermore, breast milk from the $A2^+$ mothers contained sHLA-A2 at levels averaging 36.2ng/ml resulting in milligram quantities of ingested antigen over a 3 month period of nursing alone (5). This demonstrates that oral and intravenous exposure to NIMA sHLA in the fetus and newborn is much higher than previously thought and emphasizes the importance of nursing in the overall antigen dose achieved. It is possible that this continuous exposure to low doses of NIMA in utero and orally induces a regulatory T cells that persists throughout life.

9. CONCLUSIONS

Although the exact mechanisms of the NIMA effect is not known yet, donor selection based on NIMA could be of benefit for transplantation outcome in patients for whom no HLA-identical donor is available thereby increasing the number of acceptable mismatched antigens between organ donor and recipient. Cord blood stem cell transplantation and cadaveric transplantation will undoubtedly benefit from gaining understanding of the NIMA effect as well. Discovering the mechanisms behind the NIMA effect could have implications in fields other than transplantation also, such as autoimmunity and cancer treatments.

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Footnote: In Figure 1, note that the inherited maternal antigen "B" (IMA) of recipient BD is also a NIMA from the point of view of the donor AD.

Key Words: Non-inherited Maternal Antigens, Maternal Microchimerism, T regulatory cells, Neonatal Tolerance, Transplantation Tolerance, Review

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