The influence of inherited and noninherited parental antigens on outcome after transplantation

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Abstract

Contact between the immune systems of mother and child during pregnancy has an impact on transplantation later in life. Exposure to inherited paternal HLA antigens (IPA) and the noninherited maternal HLA antigens (NIMA) can lead to either immunization or tolerization. Exposure to IPA seems to have a more immunizing effect since the mature immune system of a mother can form anti-HLA antibodies against the foreign paternal HLA molecules. On the other hand, exposure of a child to the NIMA antigens during pregnancy may lead to NIMA specific tolerance. This review provides an overview of the current knowledge on the impact of this fetal-maternal interaction on the alloimmune response and clinical transplantation.
Introduction

Kidney transplantation is the therapy of choice for patients with end stage renal failure. However, the number of grafts derived from cadaveric donors is not sufficient to overcome the need for donor kidneys. Furthermore, the high degree of polymorphism of the HLA system makes it very difficult to find a well-matched donor (1,2). Hence, more living related transplantations are performed. Graft survival is optimal when donor and recipient are HLA identical, as is the case with an HLA-identical sibling. However, in most situations this is not possible and therefore also haploidentical siblings, parents, offspring and spouses are considered as potential donors. Contact between mother and child during pregnancy can lead to either immunization or tolerization and subsequently this can have an effect on transplant outcome. A new nomenclature was proposed to assign the haplotypes of a family in which one of the siblings is a potential kidney recipient (3,4) (Figure 1). The parents or siblings that share one haplotype with the recipient and differ for the other haplotype are potential donors. The patient inherited the IMA (inherited maternal HLA antigens) haplotype from the mother and the IPA (inherited paternal HLA antigens) from the father. When the patient is transplanted with a kidney from one of the parents or from a haplo-identical sibling, the noninherited maternal HLA antigens (NIMA) or noninherited paternal HLA antigens (NIPA) are the mismatched haplotype. This scheme can also be used in case the mother or the father is the potential kidney recipient. In case the mother is transplanted with a kidney from her offspring or from her husband the IPA is the mismatched haplotype.

Figure 1. The NIMA nomenclature is patient-oriented; Children inherit one haplotype form each of the parents. Siblings of the patient share one haplotype with the recipient and the other haplotype is the noninherited haplotype. Potential donors that are HLA identical are not illustrated. The patient inherited the IMA (inherited maternal HLA antigens) haplotype from the mother and the IPA (inherited paternal HLA antigens) from the father. When the patient is transplanted with a kidney from one of the parents or from a haplo-identical sibling, the noninherited maternal HLA antigens (NIMA) or noninherited paternal HLA antigens (NIPA) are the mismatched haplotype. In case a (multi) parous mother is the potential kidney recipient, her child (or children) inherited HLA antigens from the father. These HLA antigens are called IPA. When the mother is transplanted with a kidney from either the offspring or the husband the IPA is the mismatched haplotype.
Several studies have been performed to investigate the influence of noninherited and inherited parental antigens on transplantation and both immunizing (especially IPA) and tolerizing (the NIMA effect) effects have been described. This review article will provide an overview of the current knowledge about inherited and noninherited parental antigens and their influence on transplantation.

**Inherited paternal antigens (IPA) and influence on transplant outcome**

Patients on the waiting list for kidney transplantation can be sensitised to HLA antigens through pregnancy, blood transfusion(s) and previous transplantation. The formation of antibodies directed against HLA is a major risk factor for transplant outcome. Antibodies that are directed towards the paternal HLA antigens are found in 15-30% of women that have been pregnant (5,6). The immunogenicity of paternal HLA antigens leading to antibody formation during pregnancy is determined by both the mismatched HLA antigens of the child and the HLA phenotype of the mother (7). Because contact with allogeneic (paternal) HLA antigens can lead to activation of the maternal immune system, some transplant centres have the policy never to transplant female patients with a graft that carries HLA mismatches shared by the husband.

**IPA in husband-to-wife transplantations**

Several studies have investigated the influence of IPA on transplant outcome. Terasaki et al. showed that there was no difference in transplant survival between wife-to-husband and husband-to-wife transplantations when the woman had never been pregnant (8). However, when the woman had previously been pregnant, graft survival was slightly lower in the husband-to-wife situation. A study by Berloco et al. also showed a slightly lower graft survival in husband-to-wife transplantation compared with wife-to-husband transplantation, although pregnancy was not included as a parameter in this study (9). Furthermore, a single centre study showed that the frequency of rejection episodes was similar in wife-to-husband and husband-to-wife transplantation. However, the start of the first rejection episode tended to occur earlier in husband-to-wife transplantation, while in addition steroid-resistant rejection occurred more often in husband-to-wife transplantation (10). Pollack et al. showed a correlation between an unfavourable graft outcome and sharing of immunogenic mismatched HLA-A or -B antigens between cadaveric donors and husbands of previously pregnant recipients (11). Furthermore, accelerated rejection was demonstrated especially in patients after husband-to-wife and offspring-to-mother transplantation (12). These studies suggest that in husband-to-wife transplantation a tendency toward inferior graft survival is seen in
recipients that were previously pregnant, which might be due to immunization of females to IPA, which is not detected in the serological crossmatch before transplantation.

**IPA in offspring-to-mother transplantations**

One would expect a similar difference if offspring-to-mother are compared with offspring-to-father transplantations. Studies in offspring-to-mother and offspring-to-father transplantation are less extensively performed. In 1977 Opelz and Terasaki showed that there was no difference in transplant survival of offspring grafts when the recipient was the mother or the father (13). In 1982, Terasaki found that offspring-to-mother transplantation had a one-year graft survival of 76% whereas the graft survival in offspring-to-father transplantation was 51%. The recipients were all non-transfused. The conclusion of this study was that there is no effect of pretransplant immunization towards IPA on graft survival. In contrast, it seems that there even is a beneficial effect (14). HLA haplotype sharing between mother and child during pregnancy may lead to immunomodulation as is also the case for HLA-DR shared blood transfusions (15-17). Finally, Mahanty et al. confirmed that the survival of offspring renal allografts was not different when the recipient was the mother or the father, although graft survival in multiparous woman was lower than in woman with a single pregnancy (18).

Taking the data of these studies together, immunization to IPA may play a role in case of husband-to-wife transplantation whereas no trend towards a worse graft survival could be observed in offspring-to-mother transplantation. A possible explanation may be that in offspring-to-mother transplantation sharing of HLA is present, whereas spousal donors often have more mismatches. Furthermore, it is possible that mothers become microchimeric of their child (19) facilitating graft acceptance of the child later in life.

However, one should take into consideration that in all cases a pretransplant crossmatch is performed to prevent hyperacute rejection due to donor-specific HLA alloantibodies. Transplantation is only performed when no antibodies are present, which implies a selection process in the women that finally will be transplanted.

Concerning the possible immunization towards IPA, it is important to screen and crossmatch patients before transplantation. Some women form anti-HLA antibodies after pregnancy that persist for a long period of time whereas in others the antibodies disappear. In order to detect historical sensitisation, cytotoxic T lymphocytes (CTLs) specific for paternal antigens can be determined in limiting dilution assays (20). To distinguish between high avidity (primed) and low avidity CTLs, CD8 monoclonal antibodies were added. In the presence of these CD8 antibodies, high avidity CTLs are still able to react, whereas low avidity CTLs are
Noninherited maternal HLA antigens

blocked. Strikingly, it was demonstrated that it is possible to detect primed CTLs that react with paternal antigens in both women with and without anti-HLA antibodies. Therefore the cellular test developed to detect these primed CTL can also be helpful to detect pre-sensitised women.

The influence of noninherited maternal (NIMA) antigens

Pre- and/or postnatal exposure to noninherited maternal HLA antigens (NIMA) is associated with a reduced HLA antibody formation against the NIMA (21) and a significantly better graft survival of kidney grafts from siblings (3,22) or from unrelated donors (23) who were mismatched for the NIMA haplotype compared with the NIPA haplotype later in life. Obviously, this led to the hypothesis that the exposure of a child to these antigens during pregnancy may lead to NIMA specific tolerance and was the start of more research towards the influence of NIMA on renal transplant outcome.

Clinical observations

The concept of neonatal tolerance was already described in 1945 when Owen et al. found that dizygotic bovine twins are born with a proportion of red blood cells derived from their twin (24). Hereafter, Billingham et al. showed that the injection of allogeneic cells into a newborn mouse induces lifelong immunological tolerance towards the donor (25). One year later, Owen et al. reported that rhesus D negative women pregnant of a rhesus D positive child are less likely to produce antibodies against rhesus if their mother was rhesus D positive (26). The interest in the NIMA effect disappeared until the observation that highly immunized patients were less likely to form antibodies against NIMA than against NIPA (non inherited paternal HLA antigens) (21,27). An overview of the literature regarding the NIMA effect in transplant recipients is depicted in Table 1.

The most relevant clinical finding with regard to transplantation and the NIMA effect came in 1998 when Burlingham et al. showed that the graft survival of NIMA haplotype mismatched sibling grafts is significantly better compared with NIPA haplotype mismatched sibling grafts (10 year graft survival of 77% and 49% respectively) (3). Notably, the graft survival of sibling grafts expressing the NIMA haplotype is equal to HLA identical siblings whereas the graft survival of sibling grafts expressing NIPA is similar to grafts derived from the parents. These findings were the result of a study in several transplant centres and the effect of NIMA derived form a sibling was shown in every centre separately, thereby showing that the NIMA effect was strong enough to overcome differences in immune suppression. However, the effect was especially noticed when patients were not taking cyclosporine.
A study by Smits et al. in cadaveric kidney transplant recipients compared the survival rate of grafts with a single mismatched antigen identical to the NIMA with the survival rate of grafts in which the mismatched antigen was not identical to the NIMA (23). They showed that recipients from donors mismatched for an HLA-A antigen that was identical to the NIMA had a significant better survival rate compared to recipients of grafts with no mismatches. This suggests that an active process of immune regulation is involved in the NIMA effect and that HLA class I plays a role in the NIMA-specific tolerance, as is also suggested by an earlier study that showed an unresponsive state at both the cellular and the humoral level towards maternal HLA class I antigens, even during late rejection (28).

Other studies also showed an improved graft survival when NIMA haploidentical siblings were used as bone marrow donor. Van Rood et al. described that there was significantly less graft versus host disease and an increased patient survival when NIMA haploidentical siblings were used as a donor for bone marrow transplantation (22). In contrast, this effect was not present when maternal grafts were used. Furthermore, Japanese transplant centres have successfully transplanted NIMA haplotype mismatched sibling and maternal stem cells into patients without T cell depletion (29,30). Patients and donors that were included in this protocol were all microchimeric for the mismatched haplotype. Chimerism may well be an important factor involved in the induction of NIMA specific tolerance.

Maternal versus sibling derived grafts expressing NIMA

Because of these observations, the question is raised why the survival of grafts derived from the mother is not equal to sibling grafts expressing NIMA (3). Besides the study by Burlingham et al., several other studies also showed that maternal kidney grafts have no improved graft survival (31,32). Several explanations can be given for this phenomenon. Of course, both when the mother is the donor and when the sibling expressing the NIMA haplotype is the donor, the mismatched haplotype is the NIMA. However, there are also differences. First, the shared haplotype with the recipient, in case the mother is the donor, is the IMA (inherited maternal antigens) haplotype, whereas the IPA (inherited paternal antigens) haplotype is the shared haplotype in case the sibling is the donor. Furthermore, the sibling was exposed to IMA during its fetal life, whereas the mother was exposed to IPA during adult life. It is known that a proportion of single- or multiparous women develop antibodies against IPA of the child (5,20). After transplantation it is possible that cells from the graft will recognize IPA of the recipient when the mother is the donor. However, when the sibling is the donor, the graft-derived cells will only recognize IMA that they also encountered during fetal life (and possibly during breast feeding). As it was already shown that the injection of allogeneic cells into newborn mice induces lifelong immunological tolerance towards the donor (25), one can imagine that the exposure
Noninherited maternal HLA antigens
to antigens during fetal life is a more favourable situation for the induction of tolerance than the exposure during adult life.
When the mother is the donor there is another possible disadvantage, namely that the cells derived from the mother that share the IMA haplotype, are sensitised for paternal minor Histocompatibility antigens (mHa) (33).
In contrast prenatal or perinatal recognition of mHa by the child may have a favourable effect in sibling transplantation as was suggested by the presence of mHa-specific CD8+ regulatory T cells in a tolerant kidney transplant recipient that received an HLA-identical but minor-mismatched (HA-1) kidney from her sister (34).
Furthermore there may be an important role for chimerism (35). Chimerism is determined as the co-existence of cells from two genetically distinct organisms in one individual. During pregnancy there is often an exchange of cells between mother and child, which leads to feto-maternal microchimerism; the presence of fetal haematopoietic cells in the maternal blood and vice versa (19,36). There are different ways how mother and child become chimeric: a child becomes chimeric during its fetal life that, as discussed before, is a more favourable state to become tolerant. A mother, however, becomes chimeric during adult life. Furthermore, a mother can also be chimeric of her own mother and of earlier pregnancies. All these factors may influence the immunologic responses and therefore may contribute to the fact that maternal grafts do not as good as sibling derived grafts.
Several studies suggest a functional link between chimerism and the NIMA effect. Recently, successful haematopoietic stem cell transplantations in microchimeric patients with NIMA haplotype mismatched sibling and maternal stem cells without T cell depletion have been performed (30). The stem cell donors used were also microchimeric. An important issue, however, is that the degree of chimerism differs which may have an influence on the strength of the NIMA effect, as was shown in an animal model (37). A case report described the persistence of microchimerism in a patient who was functionally tolerant of a maternal kidney allograft (28). In this particular patient, the presence of the chimeric cells was essential to downregulate the donor specific immune response in vitro. To what extent chimerism is really linked to the NIMA effect is still unclear.
A final difference between a maternal derived graft and a sibling-derived graft is the fact that a maternal derived graft can be seen as a second confrontation (the first confrontation was during pregnancy). In contrast, a graft derived from a NIMA haplotype mismatched sibling can be seen as a primary confrontation towards most of the antigens. The latter situation will be more advantageous for a beneficial immune response than the first situation and may also be an explanation for the differences seen in graft survival.
These clinical data that indicate that NIMA has an influence on the outcome in transplantation are based on statistical differences between groups of patients and cannot be extrapolated to an individual patient. Also the observation that only about half of the highly sensitised patients do not form antibodies against NIMA, whereas this was not the case for NIPA, clearly points out that the NIMA effect will not be present in every individual (38). Clarification of the factors that are favourable for the NIMA effect and herewith identifying those individuals that are sensitive for NIMA is an enormous challenge. Studies aiming at these questions also will help to understand the mechanism underlying the NIMA phenomenon.
<table>
<thead>
<tr>
<th>Research goal / Methods</th>
<th># Patients</th>
<th>Outcome</th>
<th>NIMA effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study of the posttransplant graft function of 37 patients that received a primary renal transplant and influence of retransplanting</td>
<td>37</td>
<td>Improved 3 yr graft function rate after maternal kidney transplantation in low-dose patients (50%) compared with non-low-dose patients (7%)</td>
<td>Yes</td>
<td>Campbell [13]</td>
</tr>
<tr>
<td>Determination of acceptable mismatch in patients with end-stage renal disease and PRA=10% with CDC</td>
<td>26</td>
<td>High frequency of NIMAS among sensitized mismatch</td>
<td>Yes</td>
<td>Chau [21]</td>
</tr>
<tr>
<td>Two matched responses in patients with end-stage renal disease given matched DST prior to transplantation with MLR</td>
<td>47</td>
<td>Significant association (p=0.02) between decreased MLR reactivity following DST and expression of NIMAs by cells of transplantation donor</td>
<td>Yes</td>
<td>Ford [54]</td>
</tr>
<tr>
<td>Analysis of MLR effects in renal transplantation</td>
<td>1000</td>
<td>Paired sera have a higher 3 yr graft survival (p=0.015) but non-matched grafts</td>
<td>No</td>
<td>Otsuka [50]</td>
</tr>
<tr>
<td>Analysis of MLR effects in renal transplantation</td>
<td>166</td>
<td>A better graft and patient survival at 3 yr (p=0.05) and long-term renal function in patients transplanted with a the paternal kidney compared with patients transplanted with a maternal kidney</td>
<td>No</td>
<td>Paleologou [53]</td>
</tr>
<tr>
<td>Comparison of reactivity in patients who had been exposed to HLA, HLA-DR, or HPA by DST and comparison of graft survival, number of rejection episodes and graft function in patients who also received a liver graft bearing HLA or HPA</td>
<td>213</td>
<td>No difference in specific antibody formation, graft survival and incidence of rejection episodes</td>
<td>No</td>
<td>Palma [51]</td>
</tr>
<tr>
<td>Link circulating donor cells to a functional role in human transplantation tolerance: maternal kidney transplantation after DST</td>
<td>1</td>
<td>Patient is microchimeric of HLA mismatch expressing donor cells and this is limited to the maintenance of the tolerant state of the patient</td>
<td>Yes</td>
<td>Burt [46]</td>
</tr>
<tr>
<td>Multi-center retrospective study of graft survival and rejection episodes in patients who received renal transplants from allograft donors having IMAs or NIMAs</td>
<td>285</td>
<td>Higher graft survival in recipients of kidney from haploidentical siblings expressing NIMAs compared with HLA+ (p=0.004)</td>
<td>Yes</td>
<td>Burt [43]</td>
</tr>
<tr>
<td>Comparison of renal function of kidney grafts with mismatched antigens identical to HLA in that of grafts in which the mismatched antigen was not identical to NIMAs</td>
<td>469</td>
<td>Significant better graft survival (p=0.05) for HLA-A, HLA-B, mismatched subidentical kidney grafts compared with zero HLA-A mismatches</td>
<td>Yes</td>
<td>Shafrin [31]</td>
</tr>
<tr>
<td>Comparison of outcomes of blood and marrow stem cell transplantation from maternal donors to those from paternal donors</td>
<td>94</td>
<td>At 5 years after transplantation, recipients of maternal hematopoietic cells have a higher overall survival than recipients of paternal hematopoietic cells (60% vs 38%, p=0.008) and a lower probability of non-relapse TRM (p=0.04), no difference in occurrence of severe acute GVHD and relapse of malignant leukemia</td>
<td>Yes</td>
<td>Tyan [58]</td>
</tr>
<tr>
<td>Analysis of graft failure and GVHD after non-T cell depleted bone marrow transplantsations from parental or haploidentical allograft donors</td>
<td>269</td>
<td>NIMAs vs HLA, haploidentical allograft BMT, lower rates of acute GVHD (p=0.02) higher survival in child vs. fat-to-child BMT, low chronic GVHD (p=0.012) and lower TRM (maternal BMT (p=0.009) and paternal BMT (p=0.03))</td>
<td>Yes</td>
<td>Var. Russ [21]</td>
</tr>
<tr>
<td>Haploidentical non-T cell depleted BMT in 6 patients with advanced malignancies (90% microchimerism of NIMAs)</td>
<td>5</td>
<td>Lack of severe GVHD in all patients (based on five-mismatched markers)</td>
<td>Yes</td>
<td>Saltanac</td>
</tr>
<tr>
<td>Determination of severity of patients with advanced hematologic malignancies who underwent TLA-2 antigens - or HLA-3 antigens acceptable non-T cell depleted BMT from microchimerism HLA mismatched donor</td>
<td>35</td>
<td>NIMAs mismatch in GVHD direction is associated with lower risk of severe grade III-IV acute GVHD compared with IPA (p=0.03)</td>
<td>Yes</td>
<td>Saltanac [21]</td>
</tr>
</tbody>
</table>

Abbreviation: DST, donor-specific blood transfusion; ST, stem transplantation, PRA, panel reactive antibodies, MLR, mixed lymphocyte reaction; CDC, complements dependent cytotoxicity; DCT, donor cell transplantation; GVHD, graft versus host disease; BMT, bone marrow transplantation; TRM, treatment-related mortality.
In vitro studies in healthy individuals

Another possible way to investigate the influence of NIMA are studies in healthy individuals. Table 2 depicts an overview of studies regarding the NIMA effect in healthy individuals.

A lower response towards NIMA compared with NIPA was shown when cord blood mononuclear cells (CBMC) were used as responder cells (38). However, other groups could not confirm these results (40-42).

Already in 1990, a study on peripheral blood mononuclear cells (PBMC) of healthy individuals did not show a difference when cells were stimulated with parental cells (43). Roelen et al. could not demonstrate an influence of NIMA on CTLp and HTLp frequencies when cells were stimulated with maternal or paternal cells (44). These studies only investigated the response towards parental cells. As already described, clinical studies showed that maternal renal allografts have a poorer graft survival than NIMA haplotype mismatched grafts derived from a sibling (3,31).

Therefore, we recently investigated the response towards maternal and paternal cells and towards sibling derived cells expressing NIMA versus NIPA separately (45). Again, by using several cellular techniques including MLR, Elispot analysis and FACS staining, we could not demonstrate an influence of NIMA on the cellular alloimmune response in adult healthy individuals. This is in sharp contrast with clinical data supporting the NIMA effect. One of the possibilities why we were not able to show the effect could be due to the fact that the healthy individuals are not rechallenged in vivo with the parental HLA mismatches.

Mice experiments demonstrating an influence of NIMA

Recently, a NIMA effect was also demonstrated in mice (46,47) (Table 3 gives an overview of several studies performed in animals). Andrassy et al. demonstrated the NIMA effect in a mouse model in which they showed that DBA/2 (H-2\(^d/d\)) heart allografts were accepted without any additional drug or conditional treatment by more than 50% of the NIMA\(^d\)-exposed F1 backcross (H-2\(^b/b\)) recipients (46).

Additionally, graft survival was increased in NIMA\(^d\)-exposed F1 backcross (H-2\(^b/b\)) recipients when transplanted with a skin graft from a semiallogeneic donor and not from a fully mismatched DBA/2 (H-2\(^d/d\)) donor. This indicates that the NIMA effect is MHC restricted in case of a skin graft, which is known to be a very immunogenic model, whereas the NIMA effect seems to be not MHC restricted in case of heart allografts. Furthermore they showed that breast-feeding is necessary to elicit a NIMA effect and that microchimerism was present at different levels in fully exposed (both in utero and orally) NIMA mice.
<table>
<thead>
<tr>
<th>#</th>
<th>Individuals or CE samples</th>
<th>Type of responder cells</th>
<th>Read out system</th>
<th>Stimulated with</th>
<th>Outcome</th>
<th>NIMA effect</th>
<th>References</th>
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</thead>
<tbody>
<tr>
<td>7</td>
<td>PBL</td>
<td>CTLp, CML, MLR</td>
<td>PC</td>
<td>No difference</td>
<td>Note: The number and loci of NIMA mismatches is not comparable with the number and loci of NIPA mismatches</td>
<td>No</td>
<td>Hasley [42]</td>
</tr>
<tr>
<td>37</td>
<td>PBL</td>
<td>CTLp; influence of breast-feeding</td>
<td>PC</td>
<td>Breast-feeding can downregulate the immune response against maternal HLA antigens (p&lt;0.001) and not against paternal HLA antigens</td>
<td>Yes</td>
<td>Zhang [57]</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>PBL</td>
<td>CTLp</td>
<td>PC</td>
<td>3 different CTL response patterns: 17977 no difference towards NIMA vs. NIPA, 2977 significantly higher (p&lt;0.005) towards NIMA vs. NIPA, 19077 significantly lower (p&lt;0.005) towards NIMA vs. NIPA Note: The number and loci of NIMA mismatches is not comparable with the number and loci of NIPA mismatches</td>
<td>Yes</td>
<td>Zhang [58]</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>CBMC</td>
<td>MLR</td>
<td>PC</td>
<td>No difference</td>
<td>No</td>
<td>Harris [39]</td>
<td></td>
</tr>
<tr>
<td>34</td>
<td>CBMC</td>
<td>FACs, CTLp, HTLp</td>
<td>PC</td>
<td>No difference</td>
<td>No</td>
<td>Falkenberg [40]</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>PBL</td>
<td>CTLp, HTLp</td>
<td>PC</td>
<td>No difference</td>
<td>No</td>
<td>Roelen [43]</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>CBMC</td>
<td>FACs, CTLp</td>
<td>PC</td>
<td>No difference in CTLp frequencies, but increase in NK-like regulatory CD3+CD8+ cells after stimulation with NIMA and increase in CTL-like CD3+CD8+ cells after stimulation with NIPA</td>
<td>Yes</td>
<td>Moretta [41]</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>CBMC</td>
<td>Standard MLR</td>
<td>PC</td>
<td>Lower cellular response to NIMA compared with NIPA (p=0.045) Note: Not always tested against both father and mother</td>
<td>Yes</td>
<td>Tsafir [30]</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>PBL</td>
<td>MLR, Elspot, FACs</td>
<td>PC and SC</td>
<td>No difference</td>
<td>No</td>
<td>Van den Boogerd [44]</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: CB, cord blood; CBMC, cord blood mononuclear cells; PBL, peripheral blood lymphocytes; MLR, mixed lymphocyte reaction; CTLp, cytotoxic T lymphocyte precursor; CML, cell-mediated lysis; HTLp, helper T cell precursor; FACs, fluorescence activated cell sorter; PC, parental cells; SC, staking cells.
<table>
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<th>Mouse strain</th>
<th>Outcome</th>
<th>NIMA effect</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study of immune responses to NIMA in infected rats after rechallenge with ST followed by MLR</td>
<td>RT1b/k or RT1a/k female × PVG male offspring; RT1b/k (NIMA RT1a) and RT1a/k (NIMA RT1)</td>
<td>No evidence of humoral tolerance to class I NIMA and cellular tolerance to NIMA Note: No NIMA control</td>
<td>Yes</td>
<td>Proper [39]</td>
</tr>
<tr>
<td>Investigation if the NIMA phenomenon can lead to activation of lymphocytes that are transferred from mother to child (tail skin transplantation mouse model)</td>
<td>BALB/c (H-2b) female × BDF1/F1 (H-2b/M) male offspring; H-2b/NIMA exposed mice and H-2b/H-2a mice</td>
<td>Significant prolongation of maternal skin graft survival (both in utero and orally exposed) Correlation with CD4 cells - lymphocytes (p=0.079) (not correlated with B cells) Note: No NIMA control but only Bc control</td>
<td>Yes</td>
<td>Zhang [15]</td>
</tr>
<tr>
<td>Investigation of maternal antigen exposure alone on tolerance induction to a primary vascularized skin diaphragm in mice</td>
<td>BALB/c (H-2b) female × BDF1/F1 (H-2b/b) male offspring; H-2b/NIMA exposed mice and H-2b/H-2a mice NIMA control; BL6 (H-2b) female × BDF1/F1 (H-2b/B) father</td>
<td>25% of NIMA exposed mice accept fully allogeneic H-2b/B graft (p=0.0004 vs controls) Both in utero and oral exposure required for tolerogenic effect Possible role for diaphragm Note: No NIMA effect detectable in other strain combinations</td>
<td>Yes</td>
<td>Akliluya [40]</td>
</tr>
<tr>
<td>Investigation of the influence of maternal antibodies expressing NIMA on the development of fetal and neonatal B lymphocytes (B cell receptor expression)</td>
<td>EFGF-Tg mouse (H-2b background) 1-5 months; EFGF-Tg mouse (H-12 background)</td>
<td>In H-2d-exposed fetuses NIMA-specific transgenic B cells (high affinity) are partially deleted during late gestation, unaltered cells downregulate their B cell receptor NIMA H-2d-exposed fetuses transgenic B cell present an active phenotype (low affinity for NIMA)</td>
<td>Yes</td>
<td>Varmusheh [47]</td>
</tr>
<tr>
<td>Investigation of the influence of NIMA on rejection after liver transplantation in a transgenic mouse model</td>
<td>CBA/Cd 4 TCR Tg/F, female × CBA BMJ 3 and Ks TCR Tg male offspring: NIMA (+) and NIMA (-)</td>
<td>NIMA mice display prolonged survival of allografts Reduced frequency of IFN-g and IL-2 producing cells and increase in IL-6 producing cells CD4 depletion restores acute rejection Note: No NIMA control</td>
<td>Yes</td>
<td>Akliluya [60]</td>
</tr>
<tr>
<td>Study of the impact of NIMA and FA on EMT and investigation of the mechanisms leading to tolerance in mice</td>
<td>BALB/c (H-2b) female × BDF1/F1 (H-2b/b) male offspring; H-2b/NIMA exposed mice and H-2b/H-2a mice</td>
<td>Reduced mortality and morbidity of CD4+ and CD8+ groups (p&lt;0.01 vs NIMA control) After EMT from a NIMA exposed mother met with one from an NIMA-exposed mother in chimeric setting CD4+ CD25+ Treg</td>
<td>Yes</td>
<td>Matsuda [46]</td>
</tr>
</tbody>
</table>

Abbreviations: B6; C57BL/6; BDF1; C57BL/6 × DBA/2P1; EGF: enhanced green fluorescence protein; EMT: bone marrow transplantation; ST: Sindbis virus; MLR: mixed lymphocyte reaction; Treg, regulatory T cell
Importantly, the NIMA effect was not present in several other strain combinations (Andrassy et al. personal communication), again indicating the heterogeneity in the development of NIMA specific tolerance. Additionally, in vitro experiments indicated a role especially for CD4+ cells in the NIMA effect (46). Indeed, the same group recently presented data in which they demonstrated an increase in CD4+CD25-latent-TGFb+ cells in NIMA-d-exposed mice (Molitor et al. personal communication). These "regulatory" T cells were further characterized by GITR expression and IL-10 production and were shown to be responsible for a decreased humoral response and tolerance to heart allografts. However, functional studies on the immunoregulatory capacity of these cells are still lacking.

In consistence with these results, Matsuoka et al. showed in a mouse model of bone marrow transplantation (BMT) that a BMT from a NIMA exposed child to the mother led to a reduction of the morbidity and mortality of graft-versus-host disease in an antigen-specific manner (47). In addition, an improved survival was observed. Furthermore, when CD4+CD25+ regulatory T cells were depleted from the donor inoculum the tolerogenic NIMA effect disappeared. These data together with the data from Andrassy et al. implies an important role for CD4+ regulatory T cells in establishing a NIMA effect. Matsuoka et al. also investigated the possibility that IPA may be able to induce tolerance in the mother. However, when a BMT from an IPA exposed mother to the child was performed, no reduction in graft-versus-host was observed.

Besides an influence of NIMA on the cellular immune response, the humoral immune response may also be important, especially when considering the initial finding that antibody formation in highly sensitised patients occurs less often against NIMA (21). In line with this finding, a study in mice was performed in which it was shown that NIMA influences the development of B cells (48). Vernochet et al. used B lymphocytes of mice, which recognized H-2Kk and H-2Kb MHC class I antigens with high and low affinities, respectively. They showed that NIMA specific B cells with a high affinity are partially deleted during late gestation and the non-deleted cells downregulated their B cell receptor. In contrast, NIMA specific B cells with a low affinity for NIMA were activated. These results indicate that patients that are highly immunized, but do not form antibodies to NIMA, may have B cells with a high affinity to NIMA resulting in tolerizing signals.
**Chapter 2**

**Triggering the NIMA effect: possible mechanisms**

Although the mechanism that is responsible for the induction of the NIMA effect is still not clear, several assumptions have been made. We already discussed the role of microchimerism as an important factor possibly involved in the NIMA effect (35). However, other mechanisms also have been proposed to play a role in the induction of NIMA specific tolerance (see also Figure 2).

**Soluble HLA**

Transfer of soluble HLA from child to mother and vice versa may be important for the induction of the NIMA effect. The 39 kD soluble HLA molecule lacks a transmembrane part because of a deletion in exon 5 (49). Because of this deletion, this soluble HLA can easily travel through the placental barrier. This molecule is absent in 16% of the population and heterozygous in 48% of the population, resulting in about 50% of the children carrying this allele. As already discussed, about half of the highly immunized patients do not form antibodies to NIMA. A study combining these parameters could reveal whether this molecule indeed plays a role in the prevention of antibody formation to NIMA.

![Figure 2](image-url)

**Figure 2.** Exposure to IPA and NIMA during pregnancy can lead to either immunization or tolerization. Exposure to IPA seems to have a more immunizing effect since the mature immune system of a mother can form anti-HLA antibodies against the foreign paternal HLA molecules. On the other hand, exposure of a child to the NIMA antigens during pregnancy can lead to NIMA specific tolerance.
Privileged site
Another issue is the presence of privileged sites in the human body. Privileged sites are sites where the immune system is supposed not to perform its destructive activities, for example in the brain, the eye and the uterus. When a graft is transplanted in such a site rejection will not occur (50). Studies in the eye showed a specific type of immune response: the anterior chamber-associated immune deviation (ACAI&D) which implies that when an immune response is started, no T cells that can mediate a delayed hypersensitivity will be present and furthermore no antibodies that are able to fix complement will be present. In order to establish such an environment several soluble factors are suggested to be important and especially the presence of transforming growth factor β (TGF-β) is supposed to play a central role as modulating cytokine. This cytokine can affect antigen presenting cells (APC) in such a way that they can not give a full stimulus to T cells once arrived in the secondary lymphoid organs. These APC may be able to induce regulatory T cells that can prevent or regulate the immune response to the encountered antigens. During pregnancy, the amniotic fluid is rich in TGF-β (51), creating a suitable environment for the induction of tolerance to noninherited maternal antigens.

Immune deviation
In contrast to the concept of neonatal tolerance, i.e. the development of tolerance or non-responsiveness towards antigens encountered by the innate immune system (24,25), immune deviation may be an alternative mechanism involved in the NIMA effect. In murine experiments it was demonstrated that immunization during the neonatal period can lead to a protective immune response rather than to tolerance depending on the ratio of DC, B cells and T cells (52), the antigenic dose (53) and the adjuvant that is injected together with the antigen (54). Hence, it was suggested that neonates are not immune privileged but, dependent on the type of immunization, generate Th2 or Th1 responses.

When translating the concept of immune deviation to the NIMA effect, one can suggest that the continuous exposure of the foetus towards NIMA results in both the development of a Th2 type immune response and in stimulation of T cells without a costimulatory signal by the DC, thereby inducing a tolerizing environment.

Blood transfusion effect
The immunogenetic relationship between mother and child implies sharing of one haplotype and a mismatch for the other. This is similar to the concept of the immunomodulating effect of the HLA-DR shared allogeneic blood transfusion. Therefore, this concept can be helpful in understanding the NIMA effect. It was demonstrated that patients treated with multiple pretransplant blood transfusions
had a significantly higher graft survival compared with non-transfused patients (55). Furthermore, leukocyte depleted transfusions were not associated with this effect, indicating that leucocytes are important for the beneficial outcome (56). Even more interesting is that the effect is especially seen when HLA-DR sharing between blood donor and patient is present, indicating a role for HLA class II (15,16). It is hypothesized that CD4\(^+\) Tregs that recognize a foreign peptide in the context of the shared HLA-DR molecule are induced by the blood transfusion. When these cells are rechallenged with the foreign peptide that the organ donor shares with the blood transfusion donor this will lead to downregulation of the immune response towards the graft (17). During pregnancy, HLA haplotype sharing between mother and child is present, indicating that a similar mechanism may occur; the recognition of foreign peptides in the context of shared HLA-DR. This may lead to the induction of Tregs, favouring the NIMA effect.

**Conclusion**

It is obvious that both NIMA and IPA have an influence on the outcome after transplantation. Exposure to IPA seems to have a more immunizing effect since the mature immune system of a mother can form anti-HLA antibodies against the foreign paternal HLA molecules (Figure 2). This can pose an extra risk factor when a mother is the recipient of a spousal or offspring derived graft. Careful determination of HLA alloantibodies and sensitive crossmatches before transplantation can reduce the risk of rejection. Furthermore, detection of primed CTLs directed towards paternal antigens can help to detect pre-sensitised women in which anti-HLA antibodies disappeared (20).

Both clinical and mouse studies clearly demonstrate a beneficial effect of NIMA. However, the mechanism involved in the NIMA effect is still not revealed, although CD4\(^+\)CD25\(^+\) Treg cells may play an important role (47). Once the mechanism will be revealed this will have an enormous impact on the understanding of tolerance and thus on tolerizing strategies in transplantation in general. Furthermore, it will provide extra opportunities for the selection of optimal donors in organ transplantation.

A multicenter study to determine the in vitro cellular reactivity in a large group of patients transplanted with a kidney derived from a parent and in patients transplanted with a kidney derived from a sibling may provide more information about the NIMA effect. Blood withdrawal should be performed at several time points (before and after transplantation) in order to determine the kinetics of the alloimmune response in these patients.

In conclusion, both inherited and noninherited parental antigens may affect graft survival. Further studies are necessary to determine the effect of IPA and NIMA on
Noninherited maternal HLA antigens

the alloimmune response of individual patients in order to use the presence or absence of parental HLA antigens in selecting the optimal donor for a particular patient.

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Chapter 2

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Noninherited maternal HLA antigens


