

# Importance of Pregnancy Induced Paternal HLA Antibody Production in Transplantation: Review

## Gebelikte İndüklenen Paternal HLA Antikor Üretiminin Transplantasyondaki Önemi

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**ABSTRACT** MHC molecules present peptide fragments of various organisms such as viral and bacterial peptides and foreign antigens to T lymphocytes and participate in the immune response against the infections. Furthermore, they can influence the outcome of cell and organ transplantations. HLA molecules are highly polymorphic and immunogenic. Therefore, immune systems of the organisms that encounter with foreign HLA antigens are activated and anti-HLA antibodies are produced. Pre-existing anti-HLA antibodies cause acute and hyperacute rejection in tissue and organ transplantations. Thus, detection of anti-HLA antibodies are important in transplantation. On the other hand, it is now known that not only detection but also the identification of the anti-HLA antibodies is important to estimate the result of the transplantation. In addition, understanding the reason for antibody production is also essential for a successful transplantation process. Pregnancy, transfusion and transplantation history of the patients are known as the reasons for the anti-HLA antibody production. Pregnancy is a unique transplant event and during pregnancy maternal immune system can tolerate the fetal cells. In pregnancy, as a consequence of maternal-fetal relationship, humoral immune response acts against the paternal antigens. Anti-paternal antibodies have been shown in 15-30% of pregnant women, and after 28 weeks of gestation. HLA profiles of the fetus and the mother may affect the production of the anti-paternal antibodies. In this review we focused on maternal-placental transfer, maternal tolerance and production of anti-paternal antibodies.

**Key Words:** Pregnancy; histocompatibility, maternal-fetal; reproduction; immunity, maternally-acquired

**ÖZET** Virüsler ve bakteriler gibi çeşitli organizmalardan kaynaklanan peptid parçalarını ve vücuda yabancı antijenleri T lenfositlere sunan MHC molekülleri, enfeksiyonlara karşı immün yanıtta görev almaktadır. Ayrıca hücre ve organ nakillerinin başarısında da katkıları bulunmaktadır. HLA molekülleri oldukça polimorfik ve immünojenik özelliktedir. Bu sebeple yabancı HLA antijenleriyle karşılaşan organizmalarda immün sistemin uyarılmasına ve anti-HLA antikorlarının üretilmesine sebep olmaktadır. Doku ve organ nakillerinde, önceden var olan anti-HLA antikorları akut veya hiperakut rejeksiyonların başlıca nedenlerindedir. Bu sebeple anti-HLA antikorlarının belirlenmesi nakillerde önemlidir. Öte yandan, bu antikorların sadece belirlenmesi yeterli olmamakta, ayrıca tanımlanmalarının da gerekli olduğu bilinmektedir. Başarılı bir organ nakli süreci için antikor üretiminin sebebinin bilinmesi gerektiği düşünülmektedir. HLA moleküllerine karşı anti-HLA antikorlarının oluşmasında gebelik, transfüzyonlar veya nakil öykülerinin olması etkilidir. Gebelik, eşsiz bir organ nakli örneğidir ve gebelik sırasında annenin immün sistemi fetal hücrelere karşı tolerans gösterir. Gebelikte, fetal hücrelerle ilişkiler sonucunda annede, babadan gelen antijenlere karşı humoral yanıt oluşur. Antipaternal antikorlar sağlıklı kadınların %15-30'unun gebeliği sırasında görülmüş ve gebeliğin 28. haftasından sonra tespit edilme oranının arttığı belirtilmiştir. Antipaternal antikorların oluşmasında, fetusun ve annenin HLA profilinin etkili olduğu düşünülmektedir. Bu derlemede, maternal-plasental madde transferi, maternal tolerans ve anti-paternal antikorların oluşumu üzerinde durulacaktır.

**Anahtar Kelimeler:** Gebelik; histokompatibilite, maternal-fetal; üreme; immünite, anneden edinilmiş

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## MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS AND HUMAN LEUCOCYTE ANTIGENS (MHC AND HLA)

**M**ajor Histocompatibility Complex (MHC) is one of the most polymorphic regions of the human genome that consists of 220 genes, and is almost 4 Mbp in length.<sup>1-3</sup> MHC molecules present the viral and bacterial peptide fragments and antigens to T lymphocytes, and participate in the immune response against the infections.<sup>4</sup> Furthermore, they can influence the outcome of cell and organ transplantations.<sup>1,2</sup>

Three distinct regions have been identified in MHC as class I, II and III. Class I and II are known as human leucocyte antigens (HLA) regions. HLA regions have 21 genes located on 6p21.3.<sup>1,2,5,6</sup> HLA Class I region encodes HLA-A, B, C, the non-classical HLA-E, F, G and molecules like HLA Class I as MICA and MICB (Figure 1).<sup>7</sup> Class I molecules are responsible for the presentation of peptides derived from the inner part of the cell to the CD8<sup>+</sup> T cells.<sup>1</sup> HLA Class II region is located at the centromeric end of the MHC, and consists of HLA-DR region (DRA, DRB1, DRB3, DRB4, and DRB5), HLA-DP region (DPA1 and DPB1 genes) and DQ region (DQA1 and DQB1 genes).<sup>7</sup>

HLA Class I molecules are glycoproteins which are expressed on the surface of the nucleated cells. On the other hand, HLA Class II molecules are expressed by macrophages, B cells, dendritic cells of the spleen, and the Langerhans cells of the skin.<sup>8</sup> Class III region is located between class I and II HLA regions, and includes non-HLA genes which are responsible for the immune response.<sup>1</sup> This region encodes complement cascade molecules, cytokines, tumor necrosis factor, lymphotoxins, and heat shock proteins.<sup>7</sup>

## IMPORTANCE OF THE ANTI-HLA ANTIBODIES IN TRANSPLANTATION

HLA molecules are highly polymorphic and immunogenic.<sup>9</sup> 6000 CI and 2000 CII proteins are encoded by over 10 000 nucleotide sequences. The high allelic diversity is advantageous for recognition of antigens and protection against various

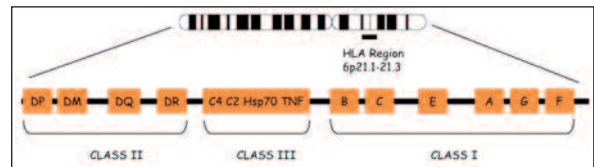


FIGURE 1: Schematic diagram of HLA gene regions.

pathogens. However, this diversity is also disadvantageous for cell and organ transplantation.<sup>10</sup> Immune response is induced in individuals who are exposed to the HLA alloantibodies. Reactivity is generated against the T lymphocytes, and anti-HLA antibodies are produced.<sup>9</sup>

HLA antibodies produced against HLA CI and CII, in other words sensitization of the patients, have several reasons such as pregnancy, transfusion, and transplantation that cause hyperacute rejections.<sup>11-13</sup> After transplantation, *de novo* alloantibodies can be generated against mismatched HLA antigens, leading to acute or chronic rejection.<sup>14</sup> Refractory response to platelet transfusion and transfusion related acute lung injury were also determined as clinical effects of HLA antibodies.<sup>15-19</sup> However, the degree and duration of the sensitization depends on the immunological history of the patient and the reason of the sensitization.<sup>20</sup>

It was detected that 20% of healthy subjects and 50% of transplantation candidates have anti-HLA antibodies. On the other hand, 30% of patients might have donor specific antibodies.<sup>10-21</sup> Rejections defined in renal, cardiac and pancreas transplantations and history of rejection differs from organ to organ.<sup>10</sup> If alloantibody formation is supported by memory T cell response, and alloantibodies against HLA antigens of donor tissues are generated, hyperacute rejection may occur in the early phase after transplantation. In case of insufficient immunosuppression, *de novo* alloantibodies against mismatched HLA antigens are generated, which may cause acute or chronic rejection. Otherwise, alloantibody-mediated rejections are resistant to immunosuppressive therapies. Therefore, histocompatibility testing and screening of the pre and post-transplantation antibodies reduce immunological risk and enable monitoring of ongoing rejection.<sup>9,14</sup>

Higher sensitization levels may cause the increase in the length of time spent on the organ waiting list.<sup>22</sup> Therefore, plasma renin activity (PRA) tests determine the organ transplantation probability of the patient whose presensitization level is low and cross-match test is found as negative before transplantation.<sup>23</sup>

The methods used for HLA antibody detection has been developed in the last 10 years.<sup>24</sup> It is known that HLA antibodies are IgG type. Thus the methods used for the anti- HLA antibody detection target IgG subunits.<sup>25</sup> Complement dependent cytotoxicity assay (CDC) is the first HLA antibody detection method established. In recent years, more sensitive and specific methods are used to detect HLA antibodies such as ELISA, flow cytometry, and Luminex technology.<sup>24</sup>

## TRANSFER OF MATERNAL ANTIBODIES THROUGH PLACENTA

Fetal and maternal circulation are separated by the placental barrier that consists of five layers; syncytiotrophoblast, cytotrophoblast, trophoblastic basal lamina, connective tissue, and fetal endothelium (Figure 2). This barrier changes during pregnancy.<sup>26</sup> Fetal-placental and maternal circulation cannot be established precisely until 10<sup>th</sup> week of pregnancy. Until this time, it is known that maternal blood diffuses through the extracellular fluid.<sup>26</sup> Furthermore, maternal antibodies can pass through the fetal capillaries via pinocytosis by syncytiotrophoblasts.<sup>27</sup>

Fetal material enters into the maternal circulation as microparticles released from syncytiotrophoblasts and spread to the maternal peripheral blood (Figure 3). In addition, fetal cells, fetal DNA and apoptotic cell debris flow into the blood circulation.<sup>15</sup> Antigen presenting cells could be triggered by any of these particles for antigen presentation.<sup>28</sup>

## MATERNAL TOLERANCE

Maternal immune system can tolerate the semi-allogenic fetal cells in pregnancy, even if maternal and paternal HLA locuses are different. However, the mechanism of this tolerance can-

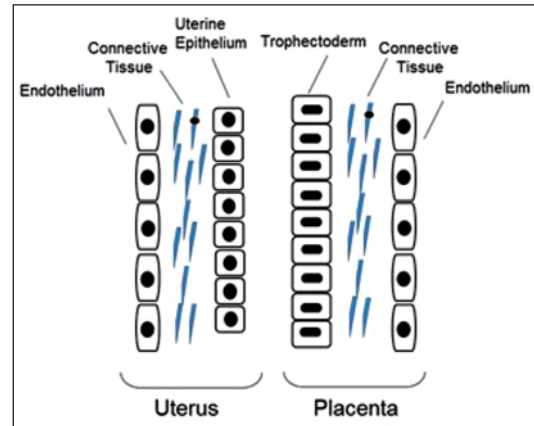


FIGURE 2: Cell layers separating maternal and fetal blood.

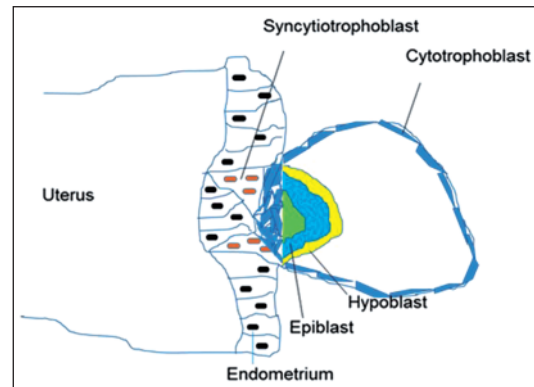


FIGURE 3: Cell layers and tissues separating maternal and fetal blood.

not be explained clearly.<sup>15,29,30</sup> This tolerance can be permanent or temporary. But it has been established that the key point of the maternal-fetal tolerance is the induction of genes that inhibit the immune response by selective inhibition of the placental genes that activate the maternal immune system.<sup>28</sup>

Recent studies have shown that the induction of the anti-HLA antibodies produced against the paternal HLA antigens has been related to foreign epitopes. Even though detectable antibody levels may decrease months or years after sensitization, naive T lymphocytes can exist for more than 10 years.<sup>15</sup>

Human trophoblast cells express only HLA-C from class Ia molecules and all 3 class Ib molecules (HLA-E, F, G).<sup>31</sup> HLA-C gene is polymorphic and if paternal antigens differ from maternal antigens, it could stimulate the maternal acquired immune response.<sup>32</sup>

In pregnant women, attack-by the maternal cytotoxic T lymphocytes is effectively thwarted by mechanisms that haven't been understood yet. Therefore, the mechanisms underlying maternal tolerance are absolutely effective. At this point, the question how maternal tolerance could be established to the semi-allogenic fetus is getting important.<sup>29</sup>

## ANTI-PATERNAL ANTIBODIES

In pregnancy as a result of the relation with fetal cells, maternal humoral immune response could be stimulated against the paternal antigens.<sup>33</sup> Antipaternal antibodies were first detected in 1958. These antibodies have been detected in 15-30% of the pregnant women by cytotoxicity testing.<sup>34</sup> However, Masson et al. detected this percentage as 54% by Luminex-based assay, and Vilches et al. detected the proportion of the immunized mothers as 84%, by single antigen bead assay.<sup>11,35</sup> However, it has been also remarked that some of the sensitization levels may have not been determined successfully, due to most patients' last pregnancy dates being long before.<sup>11</sup>

The incidence of the antibodies was shown to raise after 28<sup>th</sup> week of pregnancy.<sup>34,36</sup> This is probably due to the increased influx of fetal material into the maternal circulation in the last trimester.<sup>37,38</sup>

It is thought that production of the anti-paternal HLA antibodies depends on the level of fetal cells passing through the placenta, the HLA inconsistency between mother and fetus, or the previous pregnancies.<sup>39</sup> Furthermore, it is established that the types of HLA alleles can affect the antibody production.<sup>15</sup>

Successful pregnancies depend on developing donor-specific tolerance based on the maternal leucocyte chimerism. In rat models it was shown that maternal peripheral T cells tolerate specific paternal antigens. However, this immunologic tolerance could be temporal.<sup>31</sup> In other words the existence and level of the anti-HLA antibodies can increase or decrease, while the clinical condition is not clear.<sup>15</sup> Anti-HLA antibodies may also be detected

in nulliparous females, thus specifically in hematopoietic stem cell transplantation (HSCT) donors, the reason of which is not known.<sup>40</sup>

Complications of pregnancies occur because of the paternal anti-HLA or non-HLA antibodies or neither of them. Besides, it has been shown that anti-paternal antibodies have contributed to preeclampsia and recurrent abortions.<sup>15,41</sup>

## CONCLUSION

Pregnancy is one of the most effective sensitization events like blood transfusion and transplantation. It causes problems particularly for patients on the organ waiting list (who have chronic renal failure). The scarcity of organ donation and transplantation from cadaveric donors has led to increased number of transplantations from alive donors. Thus the success of the transplantation is becoming more important.

Women who have had pregnancy history may develop anti-HLA antibodies against the father of the fetus. Therefore, they cannot be recipients for transplantation from their partners, even if they have full HLA matching, due to the risk of graft rejection or short graft life.

Presensitization levels can be detected by cross-match testing before transplantation. But even if the cross-match test result is negative, this doesn't mean that the antibodies disappeared after pregnancy. Therefore, some transplantation centers don't perform transplantation between women who have pregnancy history and donors who have the same HLA antigens with the recipient's partner.

In some studies it was indicated that anti-HLA antibody production could be affected by the number of pregnancies and maternal-fetal-paternal HLA antigen types. The results of such studies have showed that pregnancy is a very important cause of sensitization for the women on transplant waiting list.

In conclusion, the success of the transplantation between partners depends on the comprehension of the mechanism of fetal-maternal antigen flow through placenta. Furthermore, getting a clear

alloimmunization history of the female patients, and determining the anti-HLA antibodies at a single antigen level, especially in highly sensitized pa-

tients can provide an increased chance of transplantation for female patients on the organ transplantation waiting list.

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