Mechanisms that Modify Immune Response in Neonatal Alloimmune Thrombocytopenia

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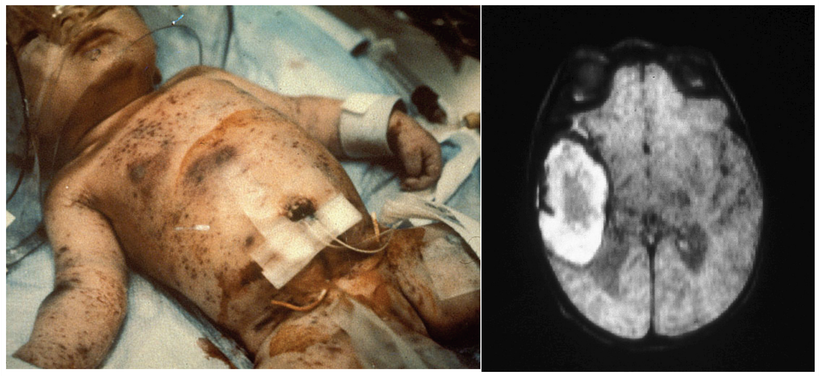
What is NAIT?

Neonatal alloimmune thrombocytopenia (NAIT) is a form of fetal and neonatal thrombocytopenia caused by maternal-fetal platelet antigen incompatibility that results in placental transfer of maternal IgG alloantibodies against the platelet antigen. Currently, there are 28 human platelet antigen systems (HPA) that are polymorphisms of various membrane glycoprotein (GP) integrins. The first HPA and most immunogenic was discovered in the 1960's and is now termed HPA-1, a diallelic system with HPA-1a and HPA-1b on the GPβ3 subunit of the fibrinogen receptor (Murphy). Approximate HPA-1 phenotype frequencies are: homozygous 1a,1a (~70%); heterozygous 1a,1b (~28%); homozygous 1b,1b (~2%).

Typically an HPA-1a negative mother (HPA-1b,1b) can develop antibodies against the HPA-1a antigen passed on to the fetus by an HPA-1a positive father. NAIT due to anti-HPA-1a often causes severe thrombocytopenia, responsible for both antenatal as well as post-natal intracranial hemorrhage (ICH) in approximately 26% of affected fetuses/neonates, as well as post-natal hemorrhage and purpura/petechiae of variable severity. ICH is the major cause of morbidity and mortality in NAIT causing blindness, significant physical and mental disability, and is fatal in 7% of affected neonates (Murphy).



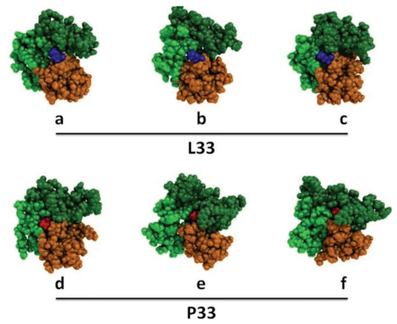
At-risk vs. Disease Disparity

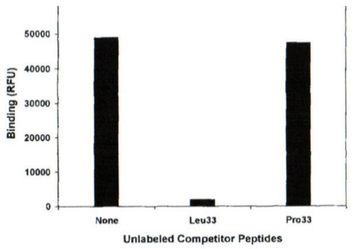
NAIT has an incidence of between 1:1000 to 1:5000 live births. Anti-HPA-1a accounts for >80% of all NAIT cases (Bassler). Since ~2% of women are HPA-1a negative (homozygous HPA-1b,1b) and ~84% of fetuses are HPA-1a positive (combined homozygous and heterozygous paternal transmission) the HPA-1a NAIT discrepant *at-risk* incidence is 1:42 pregnancies.

However only 10% of HPA-1a negative mothers produce serologically detectable anti-HPA-1. This reduced disease incidence from expected has been traced to patient specific disease modifiers. Specifically, almost all HPA-1a NAIT is found associated with a maternal HLA type DRB3*0101. HLA-DRB3*0101 has a low gene frequency of only 0.18 which has led to the theory that this HLA antigen-presenting allele is required to produce HPA-1a antibodies and explains the reduced observed incidence of clinically significant NAIT (Murphy).

Molecular Modeling and DRB Binding

The HPA-1 system is determined by a single amino acid polymorphism at position 33 of the N terminus of GPβ3 (Newman). HPA-1 antibodies bind to immunogenic conformational epitopes on the terminal 66-amino acid domain determined by numerous intrachain cysteine disulfide bridges (Bowditch).





Protein modeling revealed that the L33 allele (HPA-1a) is well exposed on GPβ3 but the P33 allele (HPA-1b) is not, as shown on the left (Jallu). Competitive binding affinity studies show a high degree of specific binding of the L33 allele, but not P33, to DRB3*0101, shown on the right (Wu). In addition, no HLA linkage has been shown for the rarely occurring anti-HPA-1b (Anani Sarab). These data explain the immunogenicity and HLA linkage of anti-HPA-1a. They also may explain the low-avidity anti-HPA-1a made by DRB3*0101 negative mothers.

Mechanism of Immunogenicity

Explanation 1: HLA-DRB3*0101 is required for all anti-HPA-1a production. Alloimmunization to HPA-1a is MHC Class II restricted.

Historically there was an associated linkage between Class II HLA-DRB3*0101 and whether an HPA-1a negative woman would make anti-HPA-1a. Maternal HLA-DRB3*0101 on APCs was highly associated with the development of anti-HPA-1a and subsequent NAIT during pregnancy, while HLA-DRB3*0101 negative women did not appear to be able to make the antibody.

Problem: Infants with NAIT from HPA-1a incompatible mothers who lack serologically detectable antibodies are sometimes born to HPA-1a negative mothers also negative for HLA-DRB3*0101 (Peterson). What is the mechanism for this rare occurrence?

Explanation 2: HPA-1a negative women lacking HLA-DRB3*0101 may still become alloimmune but make low avidity antibodies (Peterson).

Surface Plasmon Resonance (SPR) analysis is a very sensitive method to detect weak binding reactivity below normal sensitivity of serology. The maximum dilution of antibodies that can be detected by SPR is 1:1000 compared to 1:200 for flow cytometry, and the even less-sensitive standard serologic assays. SPR was able to detect anti-HPA-1a in HLA-DRB3*0101 negative mothers previously found to be negative by conventional serological testing for anti- HPA-1a.

SPR Evidence of Alloimmunization

SPR sensorgrams using IgG from 61 HPA-1a negative mothers who had no detectable antibodies (AB) by conventional serology were compared to the IgG from 30 normal subjects.

Figure 1: Shows antibodies from 18 of the samples studied using SPR produced a maximum signal against HPA-1a GPβ3 that exceeded the mean value obtained from the 30 normal subjects by more than 2 SD.

Figure 2: Sensorgrams of sera obtained from mothers with serologically detectable anti-HPA-1a that were perfused over immobilized HPA-1a-positive and negative GPβ3. Rapid rise indicates significant AB binding and slow dissociation demonstrates the strength of antibody-antigen binding.

Figure 3: Sensorgrams using IgG of HPA-1a negative mothers who were AB negative by conventional serology showed binding rates similar to mothers with detectable AB. Dissociation rates were much more rapid showing decreased AB avidity.

Figure 1

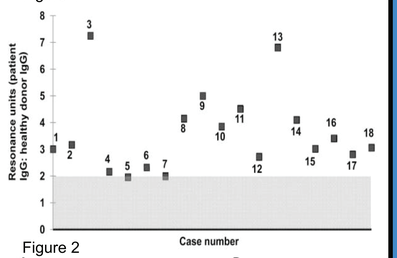


Figure 2

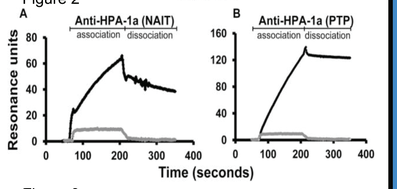
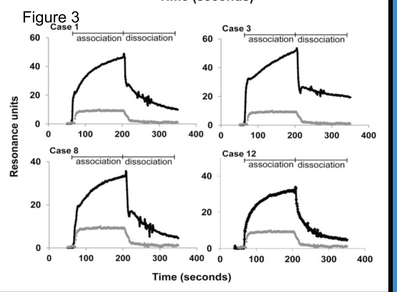


Figure 3



Conclusions

- The disparity between at-risk pregnancies and the frequency of disease presentation in HPA-1a NAIT is usually due to an HLA linked inability to make an effective antibody response
- Using molecular modeling the antigen presentation of HPA-1a allele seems to be implicated in the HLA linked response to make antibody in standard serologic methods
- Sensitive SPR data demonstrates that HPA-1a alloimmunization still occurs in HPA-1a at risk patients despite being HLA-DRB3 negative
- A monoclonal antibody that blocks HPA-1a binding (not shown) is under development in the UK to ameliorate HPA-1a mediated NAIT.

References

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