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Published in:
Clinical & Developmental Immunology

DOI:
10.1155/2012/156867

Published: 2012-01-01

Citation for published version (APA):

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Research Article

The Development of Severe Neonatal Alloimmune Thrombocytopenia due to Anti-HPA-1a Antibodies Is Correlated to Maternal ABO Genotypes

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Received 28 June 2011; Revised 16 August 2011; Accepted 16 September 2011

Academic Editor: Raivo Uibo

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Background. Maternal alloantibodies against HPA-1a can cross placenta, opsonize foetal platelets, and induce neonatal alloimmune thrombocytopenia (NAIT). In a study of 100, 448 pregnant women in Norway during 1995–2004, 10.6% of HPA-1a negative women had detectable anti-HPA-1a antibodies. Design and Methods. A possible correlation between the maternal ABO blood group phenotype, or underlying genotype, and severe thrombocytopenia in the newborn was investigated. Results. We observed that immunized women with blood group O had a lower risk of having a child with severe NAIT than women with group A; 20% with blood group O gave birth to children with severe NAIT, compared to 47% among the blood group A mothers (relative risk 0.43; 95% CI 0.25–0.75). Conclusion. The risk of severe neonatal alloimmune thrombocytopenia due to anti-HPA-1a antibodies is correlated to maternal ABO types, and this study indicates that the observation is due to genetic properties on the maternal side.

1. Introduction

Foetal-maternal incompatibility in the human platelet antigen (HPA)-1 alloantigen system is the most common underlying cause of neonatal alloimmune thrombocytopenia (NAIT), a condition where maternal alloantibodies opsonize foetal platelets during pregnancy and reduce their survival in circulation. The incompatibility is based on a single-nucleotide polymorphism (SNP) which results in a leucine/proline substitution at residue 33 in the β3 integrin that constitutes membrane glycoprotein β3 [GPIIIa] present on platelets in complex with αIIb integrin [GPIIb] [1]. On platelets, the αIIbβ3 [GPIIb/IIIa] is also the major carrier of blood group A antigen [2].

About 10% of HPA-1a negative women who have been pregnant with an HPA-1-incompatible child have detectable HPA-1a antibodies [3]. In several studies, a correlation between maternal antibody level and the severity of thrombocytopenia in the newborn has been shown [4–6]. The alloimmunization is strongly associated with the HLA-DRB3*01:01 allele [3, 7, 8]; however, only about 30% of the women with this HLA antigen are immunized. Except for the incompatibility in platelet antigen and the association to HLA, other factors which may influence the immune response to HPA-1a have not been identified.

In the present study, we have examined the maternal ABO blood groups and frequency of HPA-1a-immunization of the women identified in the large prospective screening and intervention study carried out in Norway from 1995 to 2004. We included 152 HPA-1a-immunized women, 146 of whom had altogether 158 HPA-1-incompatible pregnancies in the screening study. The ABO distribution among
immunized women was investigated, and the maternal ABO phenotype and ABO genotype was correlated to the severity of thrombocytopenia of the newborn.

2. Materials and Methods

2.1. Patients. Pregnant women were recruited for HPA-1 allotyping from three regions in Norway between December, 1995 and March, 2004 [3]. Samples for routine Rh(D) typing were also used for determining HPA-1 allotype by flow cytometry (anti-CD61 mAb), enzyme-linked immunosorbent assay (ELISA), or polymerase chain reaction (PCR) as previously described [9]. A total of 100,448 pregnant women were typed for the platelet antigen HPA-1a, and 2,111 of those were HPA-1a negative (2.1%). Of these, 1,990 were further tested, and anti-HPA-1a antibodies were detected in 154 women during the pregnancy. In total, 146 of these immunized women underwent 158 HPA-1a-incompatible pregnancies. ABO blood group typing was performed by conventional technique. Genomic typing of HPA-1 (ITGB3; rs5918 in dbSNP) and ABO in the neonates was performed in samples from cord blood or buccal swabs. For the newborns, the ABO genotype was used to predict the ABO blood group. In this context, we have defined ABO incompatibility only as an A1 phenotype in the newborn, in blood group O mothers, because individuals with A2, and the majority of those with A1 phenotype in the newborn, in blood group O

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Table 1: The maternal ABO type distribution in the pregnancies compared to the severity of NAIT.

| Maternal ABO type | Numbers of newborns with platelet count <50 × 10^9/L (within ABO type; 95% CI) | Numbers of newborns with platelet count 50–150 × 10^9/L | Numbers of newborns with platelet count >150 × 10^9/L | P value
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<tbody>
<tr>
<td>A</td>
<td>34 (46.6; 0.36–0.58)</td>
<td>13</td>
<td>26</td>
<td>0.005</td>
</tr>
<tr>
<td>O</td>
<td>12 (20.6; 0.12–0.32)</td>
<td>14</td>
<td>34</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>7 (38.9; 0.20–0.61)</td>
<td>1</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>AB</td>
<td>1 (14.3; 0.03–0.51)</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>29</td>
<td>75</td>
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Relative risk of NAIT was 0.67 (95% CI 0.48–0.94) in neonates born of women with blood group O versus blood group A.
Relative risk of NAIT was 0.43 (95% CI 0.25–0.75) in neonates born of women with blood group O versus blood group A.

Chi-square test (two-sided) for frequencies of NAIT and severe NAIT in blood group O compared to blood group A.

(restricted by lack of material). For ABO-incompatibility studies, thus only 52 of 60 blood group O mother-child pairings could be included. The fifty-two mothers with blood group O gave birth to 16 A-incompatible (blood group A1) and 36 compatible (sixteen blood group O, one B, and five A2) children. Four of the 16 A-incompatible pregnancies resulted in a newborn with severe thrombocytopenia compared to 6 of the 36 ABO-compatible pregnancies. This indicates that ABO incompatibility is not the underlying cause of the observed phenomenon reported in the present study.

3.4. The ABO Genotype of the Mothers and Platelet Counts in the Newborn. The ABO genotype of 143 HPA-1a-immunized women who gave birth to 155 HPA-1a-positive neonates was determined (data not shown). The overall O allele frequencies among the immunized women were O01 0.56, O02 0.42, and O03 0.02. Individuals with blood groups A, B, and O were further subgrouped based on genotyping, and thus, the frequencies of newborns with severe NAIT within each subgroup were compared. The cases with maternal blood group AB were excluded for further analysis due to the low number of individuals. Analysis of the platelet counts in newborns of mothers with different ABO genotypes revealed that the frequency of newborns with severe NAIT differed (Pearson Chi-square P = 0.0036) among the maternal ABO genotype groups.

Among blood group A mothers, the frequency of newborns with severe NAIT was 42% in pregnancies where the mother carried only one A allele (A101 or A201), compared to 69% where mothers carried two A alleles (relative risk 0.61; 95% CI 0.38–0.98). In pregnancies where the mother had blood group O, the frequency of newborns with severe NAIT was 9%, where the mother did not carry any O02 allele, compared to 27% where the mother carried one or two O02 alleles; however, this did not reach statistical significance (relative risk 0.33 NS P = 0.13).

Platelet counts in newborns of mothers with blood group A and O are plotted in Figure 1. The mean antibody levels between these groups were not significantly different: 11.6 IU/mL for blood group A mothers, 1.8 IU/mL for O02-negative blood group O mothers, and 11.1 IU/mL for O02-positive blood group O mothers (P = 0.18 one-way ANOVA). However, the correlation between the maternal antibody level and platelet count in the newborn for these cases was described in Killie et al. [6].

Among the NAIT cases, defined as platelet count ≤150 × 10^9/L, the mean platelet count in newborns of homozygous O01/O01 mothers was higher (83.2 × 10^9/L) than in the newborns with O02-positive mothers with blood group O (43.7 × 10^9/L) or in newborns of women with blood group A (46.1 × 10^9/L) (Table 2). Together, these data support
our hypothesis that there are genetic properties among the immunized women influencing the risk of severe NAIT in the newborn.

4. Discussion

The ABO phenotype distribution among the HPA-1a immunized women is similar to the distribution in the Norwegian population, indicating that the generation of an immune response with antibody synthesis is independent of the ABO blood group of the mother. However, we observed that whereas only 20% of pregnancies among the immunized women with blood group O resulted in severe NAIT in the newborn, 47% of the immunized women with blood group A had newborns with severe thrombocytopenia. A recent retrospective study by Bertrand et al. did not find any significant correlation between the severity of the thrombocytopenia and the ABO genotype [18]. As these authors propose, the discrepancy between Bertrand’s and our study may be due the retrospective/prospective nature of the studies. We found no indications that the low frequency of severe NAIT in the children of women with blood group O was due to ABO incompatibility between mother and foetus. Additional measurements of maternal anti-RBC IgG antibody in the women with blood group O could have given further information of any influence of potential antibodies directed against the A antigen carried by αIIb on platelets. Another hypothesis that could explain the lower frequency of newborns with severe NAIT among the immunized mothers with blood group O, compared to blood group A, is that the ABO gene is located close to a gene encoding an immunoregulatory factor with polymorphic variants. In order to approach this question, we compared NAIT to ABO genotypes. The allelic differences in the gene encoding the A/B glycosyltransferases are defined by SNPs that changes the amino acid sequence of the enzyme and thereby its glycosylating properties. The A101, A201, B101, O01, O02, and O03 alleles all produce transcripts (although A transcripts are virtually undetectable in peripheral blood) [19, 20], but the O01 and O02 transcripts both contain a shift in the reading frames that will severely truncate any resulting protein and leave it without enzymatic activity [21]. It is still unclear if these short nonfunctional proteins are expressed at all although it has been suggested [22].

The ABO genotype frequencies in the Norwegian population are not known, but the O allele frequencies observed in immunized women are similar to the frequencies reported for a Swedish population [15], where the O02 constitutes about 40% of all O alleles and O03 allele is infrequent. This further shows that the generation of an immune response to HPA-1a is independent of ABO blood groups. However, when it comes to development of NAIT, the different risks of severe thrombocytopenia observed in genetic subgroups of blood group A support the hypothesis that a genetic linkage may be involved, rather than the ABO phenotype itself even though the mechanism is still not understood. Although the differences in the O02-positive and O02-negative subgroups of blood group O do not reach statistical significance, an interesting trend is observed.

Phylogenetic analyses of the ABO locus have shown that the O02 probably is an ancient allelic lineage at the ABO locus, separate from the A101 and O01 alleles [23]. Therefore, it is interesting to subdivide the blood group O women according to their genotype. The 9q34 chromosomal region, where the ABO gene is located [24], contains several loci encoding immune response regulating genes. There is obviously no genetic linkage between the ABO [9q34] and ITGB3 [17q21] loci. The association of the ABO type to the development of severe NAIT could be due to a potential linkage to one or more gene(s) encoding regulatory factors. Further investigation has to be conducted to find out whether such factors are linked to the ABO locus in a way that can explain our observation.

5. Conclusions

The development of severe NAIT in newborns is caused by transfer of platelet-reactive antibodies during pregnancy; however, several biological factors likely play a role in the immune response mechanism. In the present study, with data from a prospective NAIT study, we showed that the risk of severe NAIT due to anti-HPA-1a antibodies is correlated to maternal ABO types. The results indicate that there are genetic properties related to the maternal ABO genotype that influence the immune response that cause severe thrombocytopenia in the newborn of anti-HPA-1a immunized mothers.

Authors’ Contribution

B. Skogen was responsible for conception of the study. M. T. Ahlen contributed to study design, performed the experiments, collected data, and performed statistical analyses. A. Husebekk supervised the research; M. L. Olsson...
contributed with study design and interpretation of ABO analyses; J. Kjeldsen-Kragh and M. K. Killie contributed to study design and interpretation of data, the paper was written by M. T. Ahlen and B. Skogen, with contributions from A. Husebekk, J. Kjeldsen-Kragh, M. K. Killie, and M. L. Olsson. All authors critically reviewed the paper and approved the paper for publication.

**Conflicts of Interests**

The authors reported no potential conflicts of interest.

**Acknowledgments**

The authors would like to thank Dr. Åsa Hellberg for help with technical advice regarding ABO genotyping. This work was supported by a Grant from the North Norway Regional Health Authority (Bodø, Norway).

**References**


