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Murine models for studying treatment, prevention and pathogenesis of FNAIT

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ABSTRACT

Maternal alloimmunization to paternally inherited antigens on fetal/neonatal platelets can cause fetal/neonatal alloimmune thrombocytopenia (FNAIT) after antibody-mediated removal of platelets from the fetal circulation. The complications vary from mild bleeding symptoms to severe intracranial hemorrhage and subsequent neurological impairment or death. Studies on *in vivo* mechanisms are challenging to measure directly in pregnant women, rendering murine models as valuable and attractive alternatives, despite some critical differences between mice and men affecting the translational value. Here we present and discuss, the different murine models that substantially have increased our knowledge and understanding of FNAIT pathogenesis – as well as preclinical evaluation of therapeutic and preventive strategies.

1. Introduction

Fetal/neonatal alloimmune thrombocytopenia (FNAIT) is a condition caused by maternal alloimmunization against paternally inherited platelet antigens on fetal/neonatal platelets, and subsequent antibody-mediated removal of platelets from the circulation of the fetus or newborn.

Alloantigens are formed due to allelic variations of glycoprotein complexes important for hemostasis, by their function as receptors for fibrinogen, fibronectin, vitronectin, collagen and von Willebrand factor (vWf). The human platelet antigens (HPA) that may cause alloimmune complications have been designated into 35 systems (HPA-1 to -35) by the International Platelet Immunology Nomenclature Committee of the International Society of Blood Transfusion (ISBT). An updated overview is available in the HPA database at http://www.versiti.org/HPA.

The GPIIb/IIIa (αIIbβ3 or CD41/CD61) complex is a heterodimeric αβ integrin (encoded by *ITGA2B* and *ITGB3*) that form a receptor for fibrinogen, fibronectin, vitronectin and vWf, and most of the HPA systems are present on this complex. Alloimmunization to HPA-1a cause the vast majority of FNAIT (~80 %) in Caucasians [1] whereas antibodies to HPA-4 antigens are more frequently seen in the Asian population. Other HPA determinants that cause FNAIT are located on the GPIb-IX-V complex, the GPIa/IIa complex and the CD109 protein on the platelet surface. In addition, isoantibodies to CD36 in CD36-deficient individuals are also a cause of FNAIT [2,3].

In humans, maternal IgG antibodies are transported across the placental membranes and into the fetal circulation by Fc receptor-mediated mechanisms that provides the fetus with humoral protection during gestation and the first 3–6 months after birth while the endogenous immune system is developing and maturing. This transport of antibodies has been studied in detail the last decades, and has been shown to be effectuated by the neonatal Fc receptor (FcRn). This receptor is not exclusively expressed on human syncytiotrophoblasts in placental tissue, but importantly also on endothelial cells and in epithelial tissue. FcRn is also crucial for the significantly longer half-life of IgG compared to the other immunoglobulin classes by pH-dependent recycling [4]. Even though FNAIT is defined as thrombocytopenia with or without bleeding in the fetus/newborn, the complications due to potential harmful effect of the antibodies towards antigen-epitopes expressed on placental tissue is in the spotlight after observations of low birthweight in babies of HPA-1a alloimmunized mothers [5]. Today, there are no licensed drugs for neither prevention nor safe and effective treatment of FNAIT. However, there is an increasing trend for off-label use of human intravenous IgG (IVIG) treatment of women with anti-HPA-1a antibodies, and many clinical guidelines recommend IVIG treatment of women with a known obstetric history of severe FNAIT with intracranial hemorrhage (ICH) [6].

Knowledge about genetic and molecular basis, pathogenesis and prevalence of FNAIT has greatly improved the last 30 years, both due to the improved repertoire of laboratory analyses, and a number of

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retrospective and prospective human studies. However, studies on *in vivo* mechanisms are challenging to measure directly in pregnant women and fetuses, rendering murine models as valuable and attractive alternatives. To date, several different models have been used for studying FNAIT. Here, we present and discuss the insights previous and current models have given.

2. Similarities and differences - man *versus* mice relevant for FNAIT

In order to translate results from murine experiments to the human setting, the differences between several facets of FNAIT biology should be carefully acknowledged. These factors include differences in hematology, pregnancy-related anatomy, and immunization: antigen source, antigen presentation and immune cell interactions, but also antibody-related factors as transplacental transfer, platelet opsonization and removal as well as effect on endothelial cells in the vascular system and bleedings.

2.1. Human versus murine platelets and platelet integrins

The murine platelets are smaller and their life-span of 3–4 days is markedly shorter than the 8–12 days in humans [7]. Platelet counts in inbred mice, show variations between strains, age and gender, with reports of normal counts in the range of $600-1500 \times 10^3/\mu L$, with most commonly reported platelet count in the range of $900-1200 \times 10^3/\mu L$ [8–12]. The platelet count in pups is roughly doubling from day of delivery ($\sim 400-600 \times 10^3/\mu L$) during the first two weeks of life, to adult levels within 8 weeks [9]. Thus, the baseline platelet count should be determined carefully for every model, keeping in mind that there are also variations in the observed platelet counts due to differences in sampling techniques (sampling point, sampling method, platelet activation and clotting) and quantitation methods (manual counting, automatic analyzers or flow cytometry) [10,11]. Severity of thrombocytopenia in or between FNAIT models based on platelet counts alone should be compared with caution.

Platelet integrin complexes function as receptors for hemostatic factors important for platelet function and hemostasis in mice as in humans. Amino acid sequence identity between human and murine platelet integrins are generally more than 80 % for the mature proteins (not including the signal peptide, based on NCBI reference sequences): 0.91 for GPIIIa (*ITGB3*), 0.80 for GPIIb (*ITGA2B*), 0.93 for GPIbα (*ITGB1*) and 0.83 for GPIa (*ITGA2*) in line with original characterizations [13,14]. High amino acid sequence identity score may however, not necessarily represent epitope homology – depending on the conformational nature of the given epitope. Since there are no reports of alloantigenic platelet integrin variants between the inbred strains, immunogenicity is created either by knockout of murine glycoprotein genes or by using human platelets. Human anti-HPA-1a antisera do not bind the murine wild-type GPIIIa, which otherwise would have precluded all use with such antibodies with the mouse as a host [15]. Thus, for studies of antibodies reactive with the human GPIIIa (their reactivity patterns, *in vivo* effect and treatment potential), chimeric models, recombinant proteins, or murine platelets, with reconstructed human epitopes are required.

2.2. Human versus murine placenta and placental transport

The placenta is of high evolutionary diversity among species, and for some research areas (placental disease/toxicology studies), the murine placenta has been demonstrated to have less translational value due to its differences in major key factors for placentation and placental function, reviewed by Schmidt et al. [16].

In mice, the pups are growing in multiple embryonic sacs in each of the two uterine branches, where each embryonic sac contains a single pup and its own placenta. Importantly, for models of alloimmunization, both the human and mouse have discoidal, hemochorial placentas where maternal blood are in direct contact with the trophoblast cells (of fetal origin). Maternal immune cells may interact with fetal antigens expressed on the outer cell layer in the intravillous space in humans, which correspond to the structurally different placental labyrinth in mice [16]. In addition to the chorioallantoic placenta, the mouse also has a secondary placental structure - the inverted yolk sac - that surrounds the fetus throughout gestation, and is the structure that allows transfer of maternal antibodies to the fetus by transcytosis by the FcRn in the inner layer of the inverted yolk sac structure [17]. Thus, transplacental transport of antibodies is mediated by anatomically different structures in humans and mice, while facilitated by the same receptor.

3. Mouse models in FNAIT – Insights from different models

Different mice strains demonstrate diverse immune responses due to variations in genetic background [18,19]. Alloantigens, that in most cases differ from self by a single amino acid residue substitution, have a limited number of antigenic peptides to be presented in MHC class II, compared to other foreign substances as microbes, parasites and viruses. Therefore, differences in antigen-presenting capacity due to different MHC haplotypes may affect immunization in this setting. In addition, it should be kept in mind that the uniformity within the mouse colony due to genetic identity within an inbred mice strain, dramatically changes whenever an antigen-specific immune response is mounted by the adaptive immune cells, due to its nature of random sequence generation – affecting the potency, titer and polyclonality of antibody repertoire.

The choice of mouse strain or design of knockout models may significantly influence the results of the study, as their intrinsic phenotype may affect their overall health, relevant hematology, immune responses and breeding.

3.1. Murine model designs

In 2006, Ni and colleagues presented a model of GPIIIa knockout ($\beta3^{-/-}$) mice backcrossed onto a BALB/c background [20]. The original β3-deficient mouse strain was designed by Richard O. Hynes and his group to study Glanzmann thrombasthenia [21], and due to its suitability to study platelet conditions it was utilized to generate the first murine model of FNAIT [20]. Use of a corresponding GPIbα-knockout model, originally made to study Bernard-Soulier syndrome [22], was also reported by Li and colleagues [23]. In these two models, alloimmunization (or rather isoimmunization) is achieved by repeated injection of wild-type platelets, and pre-immunized females are bred with wild-type males to assess the effect of the antibodies on pregnancy and pups. Platelet glycoprotein-deficient mice are however phenotypically affected: GPIIIa-deficient mice have higher fetal/neonatal mortality rate due to placental defects, anemia and hemorrhages, and by design impaired platelet function [21], whereas GPIbα-deficient mice have reduced platelet count and reduced platelet function [22]. A chimeric model expressing human GPIIIa (originally used to study Glanzmann thrombasthenia [24]) allows studies on human anti-GPIIIa antibodies in a murine system [25].

For other studies, non-obese diabetic/severe combined immunodeficient (NOD/SCID) or SCID mice has been used. These mice harbor the SCID mutations leading to mice without functional B and T cells and the NOD mutations leading to mice without 1) functional natural killer (NK) cells, 2) functional antigen-presenting cells and 3) circulating complement; the mice will be affected in many ways [26]. In this model, the mice functions as a host, injected with human material (antigen and antibodies), but may nevertheless depend on immune-related effects like Fcγ receptor-mediated functions. The knowledge acquired from all these models, with respect to treatment, prevention and pathogenesis is discussed below.

3.2. Studies on therapeutic strategies

3.2.1. Therapeutic administration of IVIG to treat FNAIT

In the GPIIIa knockout model, repeated injections of wild-type platelets into $\beta3^{-/-}$ mice gave robust and potent generation of β3 integrin-specific antibodies, which subsequently caused thrombocytopenia when injected into wild-type mice [20]. The titer of maternal antibody against β3 integrin correlated with the severity of FNAIT in the pups, in pre-immunized females bred with wild-type males. High antibody titer caused severe FNAIT with signs of bleeding, ICH, and in the most severe cases miscarriage and increased mortality rate [20]. This model, resembling human anti-GPIIIa-mediated FNAIT conditions, allows studies of the pathogenic mechanisms as well as studies attempting to interfere with pathogenesis. IVIG is licensed for use to treat a number of immune-deficient conditions, but is also used to treat autoimmune diseases. Human IVIG has also been used in mouse models of immune thrombocytopenia (ITP) [27]. By applying this strategy to the above FNAIT model, it was shown that administration of IVIG in pre-immunized $\beta3^{-/-}$ mice (bred with male wild-type mice) during pregnancy, increased platelet counts of the newborn pups, and more importantly, bleeding symptoms, pup mortality and number of miscarriages were diminished [20]. The therapeutic effect of IVIG was further confirmed in a number of other, subsequent studies mentioned below.

3.2.2. FcRn – a potential therapeutic target

The transplacental transport of maternal antibodies is a central part of human FNAIT pathogenesis. In 2010, Ni and colleagues reported another mouse model, generated through the combination of β3-deficient mice and FcRn-deficient mice, creating $\beta3^{-/-}FcRn^{-/-}$ mice [28]. By pre-immunizing and breeding deficient and wild-type β3/FcRn mice in different combinations, they showed that also in this murine model, fetal rather than maternal FcRn, is crucial for transportation of antibodies across the placenta and thus, critical for the development of FNAIT. When pre-immunized $\beta3^{-/-}FcRn^{-/-}$ females were bred with $\beta3^{-/-}FcRn^{-/-}$ males, no FNAIT occurred in the pups even though anti-β3 antibodies were present in the mother. However, by treating these pregnant mice with IVIG, there was a decrease in antibody titer in the mothers, showing that IVIG also may function through FcRn-independent pathways [28]. In the light these results, they targeted this receptor to reduce harmful effect of anti-β3 antibodies, as had previously been done in animal models of autoimmune conditions [29]. Indeed, they were able to show that by injecting an anti-FcRn antibody into pregnant pre-immunized $\beta3^{+/+}FcRn^{+/+}$ (bred with wild-type males; $\beta3^{+/+}FcRn^{+/+}$) the newborn pups had normal platelet counts and showed no abnormalities. Less severe thrombocytopenia was also seen in pups when treating pregnant pre-immunized $\beta3^{-/-}FcRn^{-/-}$ mice (bred with wild-type males) with anti-FcRn antibody [28]. As the authors pointed out, the monoclonal anti-FcRn antibody could be administrated with good effect in lower doses than IVIG. Thus, an anti-FcRn antibody could be an attractive alternative to IVIG which is a plasma-derived product of limited source, has batch-to-batch variations, and has adverse effects when administered in high dose.

Noteworthy, when comparing FcRn between species, human FcRn shows immense restrictions when binding to IgG from different species with no or very low binding to murine IgG, while murine FcRn binds human IgG better than murine IgG [30]. Andersen and colleagues also confirmed this in an *in vitro* experiment using soluble recombinant murine and human FcRn binding to IgG1 [31]. These cross-species differences may affect the experimental results of half-life and transplacental transfer analyses as well as potential mechanistic artifacts in the model [32]. Several anti-FcRn antibodies are in the drug development pipeline for autoimmune and alloimmune conditions. Of utmost relevance to FNAIT, the M281, a human anti-FcRn antibody has been demonstrated to efficiently reduce transplacental transport of IgG in a human *ex vivo* placental perfusion model [33]. This molecule is currently in clinical phase trials as a potential treatment for the alloimmune complication hemolytic disease of fetus and newborn [34,35].

3.2.3. FNAIT treatment by prevention of fetal platelet destruction

A chimeric mouse model expressing a hybrid complex of murine αIIb/human β3 [24] was used by Ghevaert and colleagues to examine the ability of the recombinant IgG1 antibody B2G1 (HPA-1a-specific mAb) to prevent platelet destruction [25]. Using bone marrow from β3-deficient mice [21], they transduced it with a lentivirus vector containing cDNA of the human *ITGB3* comprising either HPA-1a or 1b, and next, the bone marrow was transplanted into lethally irradiated β3-deficient mice [24,25]. Interestingly, $F(ab')_2$ B2G1 antibody fragments was used as a proof of principle for the effect of non-FcγR-binding reagents to inhibit and prevent platelet destruction by intact B2G1 antibody or polyclonal anti-HPA-1a sera in the chimeric mouse model [25]. Consequently, a modified B2G1 variant without FcγR-binding can have platelet protective effects and thus potentially be a candidate for FNAIT treatment, given proper FcRn-binding for transport to the fetus.

Another mouse model was presented in 2009 by Bakchoul and colleagues, in an FNAIT study focusing on platelet clearance in NOD/SCID mice (JAX stock #001303) [36]. This immunodeficient mouse model does not have functional lymphoid cells and have no or low levels of immunoglobulin making the model ideal for graft studies [26] but also antibody-mediated platelet clearance studies [37]. The injection of maternally derived human anti-HPA-1a IgG after supplying NOD/SCID mice with resting human platelets from HPA-1ab donors, mediated clearance of these platelets *in vivo* [36]. By introducing a monoclonal murine antibody (mAb SZ21, $F(ab')_2$) directly after platelet transfusion, the platelet clearance by human anti-HPA-1a antibodies was efficiently inhibited. Importantly, this study confirmed the potential to treat FNAIT using agents that block the binding of the maternally derived antibodies to the HPA-1a epitope [36]. The SZ21 antibody was later deglycosylated by Bakchoul and colleagues and injected into pregnant BALB/c mice, where it was transferred to the fetus to the same degree as native SZ21, and still blocked the binding of maternal antibodies to the HPA-1a epitope [38]. As deglycosylation of IgG antibodies is well known to terminate Fc receptor-binding and thus Fc-related effector functions [39], it could make the antibody suitable for treatment [38]. Thus, this study supports that deglycosylated monoclonal antibodies could be a potential FNAIT treatment in the future.

3.2.4. Potential therapeutic targeting of T cells by peptides

There has also been interest in targeting the T cell response in FNAIT, as the peptide harboring the HPA-1a epitope (the Leu33 residue) is efficiently presented to HPA-1a-specific T cells by antigen-presenting cells expressing the DRA/DRB3*01:01 molecule [40–46]. Jackson and colleagues made attempts to dampen already established immune responses by tolerization with peptide treatment [47]. In a SCID mice model depleted of NK cells and macrophages, PBMC from *HLA-DRB3*01:01*-positive HPA-1a alloimmunized women were intraperitoneally injected followed by administration of HPA-1a-derived peptides. After 2 and 3 weeks, $HPA\text{-}1a^{+}$ platelets were injected to boost any present response against the antigen. However, in this system, no consistent effect of this regimen was seen, measured by anti-HPA-1a antibody responses *in vivo* [47]. This kind of therapeutic strategy still holds potential, but may require additional factors targeting the antigen-presenting cells, other than peptide alone, to induce an effective tolerogenic effect on T cells [48].

3.3. Potential prevention of alloimmuniztion

While many studies are designed to study treatments of FNAIT, the notion that immunization often takes place in connection with delivery [49], opened the possibility to employ the prophylactic strategy that has successfully reduced the incidence of RhD alloimmunizations. The

administration of human plasma-derived anti-D antibody treatment is based on the concept of antibody-mediated immune suppression (AMIS). Still it is unclear exactly how passively injected antibodies suppress the immune system, but different mechanisms are proposed, as discussed by Kumpel and Elson [50]. Briefly, this includes inhibition of B cells through crosslinking of the B-cell receptor and the inhibitory receptor FcγRIIB on B cells with the antibody-antigen complex, or clearance of antigens, where the antigen is removed from the circulation before the antigen is seen and processed by the immune system [51]. However, Bernardo and colleagues could induce AMIS in knockout mice for either activating or inhibitory Fcγ receptors [52]. AMIS was similarly induced in these models when using antibodies lacking their Fc region indicating that AMIS also works through Fc and Fcγ receptor-independent pathways [52]. In addition, antibody masking of the epitope that blocks the recognition of antigen by B-cell receptors has been suggested as another possibility. However, based on stoichiometric calculations only a small portion of the RhD epitopes would be masked [50].

In investigation of the potential prophylactic approach, Tiller and colleagues managed to induce AMIS by injection of mouse anti-β3 antisera subsequent to transfusion of murine wild-type platelets into $\beta3^{-/-}$ mice [53]. Importantly, AMIS was also seen using human anti-HPA-1a antibodies or the monoclonal murine antibody SZ21 after transfusion of human HPA-$1a^{+}$ platelets [53]. Thus, the findings from this study supported the concept that also FNAIT may be prevented through a prophylactic regimen.

3.4. Insights into pathogenesis from murine models

3.4.1. Anti-GPIbα-antibodies may cause miscarriages through reduced placental function

Although the majority of FNAIT complications are due to αIIbβ3 incompatibilities, there are also cases reported due to alloantibodies against GPIbα [1]. By making a GPIbα knockout mouse model similar to the GPIIIa knockout, Li and colleagues showed that pre-immunized GPIbα-deficient female mice have an very high miscarriage rate when bred with wild-type mice compared to the β3-deficient mouse model [23]. The miscarriages were accompanied by reduced placental function and development, while treatment with either anti-FcRn antibodies or IVIG successfully increased the number of live pups. This study suggests that there might be many unreported FNAIT incidents due to frequent miscarriages caused by antibodies against glycoprotein GPIbα, which then masks the severity of this condition. Nevertheless, this study also supports the use of IVIG or antibodies against FcRn as FNAIT treatment [23].

In a follow-up study, it was shown that immunogenicity of GPIbα is lower than for GPIIIa in these knockout models, as more wild-type platelets were required for potent antibody responses in immunization experiments [54]. Mounting an efficient antibody response to foreign structures generally depends on activation of dendritic cells and induction of a pro-inflammatory environment, by binding of pattern recognition receptors to microbe-specific structures and/or endogenous stress signals. Allogenic proteins, such as blood type antigens and platelet glycoproteins will most likely not activate the innate immune cells by their sole presence in circulation. It was previously reported that ongoing authentic infection, or the mimicking of inflammation with Poly I:C can induce or enhance antibody responses in RBC-alloimmunization models [55]. Accordingly, injection of LPS or Poly I:C (mimicking bacterial or viral infections, respectively) both enhanced antibody production and subsequent miscarriages in the knockout mice [54]. This is adding a piece to the puzzle that factors like inflammation may influence immunization and the quality and quantity of the alloreactive response. Whether the timing of the inflammation signals are critical for immunization to platelet antigens, as was indicated in a mouse model where the timing of Toll-like receptor 3-induced inflammation and exposure to RBC antigens affects the degree of alloimmunization [56], is not yet known.

3.4.2. Intracranial hemorrhage might be caused by impaired angiogenesis

The last decades, the focus of FNAIT treatment has been on improving the platelet count of the fetus or neonate, but in a recent study by Yougbaré and colleagues, they highlight that β3 integrin has an important role in angiogenesis in relation to development of the fetus [57]. Here, they showed that it might not be thrombocytopenia that causes ICH, but rather the maternal anti-β3 antibodies that impair angiogenesis leading to ICH in the fetus. In the β3-deficient mouse model, anti-β3 antibodies impaired the vessel density in the brain and retina of the delivered pups, also accompanied by increased endothelial cell apoptosis and inhibited angiogenic signaling. Importantly, anti-HPA-1a antibodies of anti-αvβ3 specificity in development of FNAIT-associated ICH, mediated through binding to endothelial cells, have also been reported [58]. As shown previously [20,23,28], IVIG can be used to ameliorate FNAIT, but in this study they also saw improvement of vascular density and less apoptosis [57]. These findings indicate that treatment of FNAIT preferentially should be focused on eliminating the maternal anti-β3 antibodies rather than increasing the platelet count of the fetus. Another study using the β3-deficient mouse model recently found that activated NK cells causes apoptosis of β3 integrin-positive trophoblasts in the placenta, which subsequently leads to placental dysfunction and miscarriages [59]. By depleting the NK cells or through functional blockage of the NK receptors FcγRIIIa or NKp46, both placental growth restriction and miscarriage were reduced [59]. This study introduces another alternative approach to treat FNAIT, but in addition, it also sheds light on the pathological mechanisms in the development of FNAIT.

3.4.3. Clinical significance of low-avidity anti-platelet antibodies

Bakchoul and colleagues used the NOD/SCID model to show that low-avidity anti-HPA-1a antibodies from a series of FNAIT cases cleared human platelets from HPA-1ab donors *in vivo* - even though these antibodies may not be detected in standard serological and solid-phase glycoprotein-specific assays [60]. Such antibodies can be detected by surface plasmon resonance (SPR) measurements which allows increased sensitivity in part by eliminating the washing steps used in conventional methods [60].

Peterson and colleagues later confirmed (using the NOD/SCID mice model) that low-avidity antibodies can cause FNAIT and that many cases go undetected [61]. In this study, most women that had low-avidity antibodies only, were *HLA-DRB3*01:01*-negative suggesting that these women have a predisposition to generate such antibodies [61]. Tentatively, this could be due to a less efficient underlying T cell response supporting potent antibody responses, as several reports support the role of *HLA-DRB3*01:01* in HPA-1a antigen presentation [40–45] and clinical outcome [46,62].

3.4.4. Using the murine system to study the antigenic epitopes for antibodies

Comparative analyses to study antibody reactivity have used human and murine recombinant proteins. Based on amino acid sequence comparisons, residues important for anti-HPA-1a antibody binding was determined two decades ago [15]. Since then, detailed characterizations of the glycoprotein complex and conformation important for antibody binding have been resolved by crystal structures and experiments with site-directed mutagenesis variants [63–65]. However, due to recent CRISPR/Cas9 technology for site-directed mutagenesis in the murine genome, mouse platelets are now valuable again: designed, humanized murine platelets to map the epitope for different anti-HPA-1a monoclonal antibodies [66].

4. Conclusions and future perspectives

Important insights into the pathogenesis, treatment and prevention of FNAIT have been given by the elegant studies on knockout mice models. An advantage with knockout models is that injections with wild-type antigen causes immunization against the entire native

protein, likely representing a more general strategy, not restricting the translational value to a single alloantigen system. However, from a molecular interaction point of view, the glycoprotein knockout models unfortunately lack the essential fine-tuned balance of reactivity of self and non-self, both regarding conformational epitopes for antibody-specificity and for peptide-antigen presentation and recognition by antigen-specific T cells. Most antibodies causing FNAIT are thought to be specific to a single epitope, at least caused by a single amino acid substitution. Thus, the study of preventive or therapeutic treatments using monoclonal antibodies could be more challenging to examine in knockout mice. Models that take these factors into account, would better mimic the epitope-specific responses in future studies, as improved strategies are needed for diagnosis and treatment of women with a potential high risk of FNAIT in their newborns.

Declaration of Competing Interest

None.

References

[1] Davoren A, Curtis BR, Aster RH, McFarland JG. Human platelet antigen-specific alloantibodies implicated in 1162 cases of neonatal alloimmune thrombocytopenia. Transfusion 2004;44:1220–5.

[2] Curtis BR, Ali S, Glazier AM, Ebert DD, Aitman TJ, Aster RH. Isoimmunization against CD36 (glycoprotein IV): description of four cases of neonatal isoimmune thrombocytopenia and brief review of the literature. Transfusion 2002;42:1173–9.

[3] Lin M, Xu X, Lee H-L, Liang D-C, Santoso S. Fetal/neonatal alloimmune thrombocytopenia due to anti-CD36 antibodies: antibody evaluations by CD36-transfected cell lines. Transfusion 2018;58:189–95.

[4] Pyzik M, Sand KMK, Hubbard JJ, Andersen JT, Sandlie I, Blumberg RS. The neonatal Fc receptor (FcRn): a misnomer? Front Immunol 2019;10.

[5] Tiller H, Killie MK, Husebekk A, Skogen B, Ni H, Kjeldsen-Kragh J, et al. Platelet antibodies and fetal growth: maternal antibodies against fetal platelet antigen 1a are strongly associated with reduced birthweight in boys. Acta Obstet Gynecol Scand 2012;91:79–86.

[6] Lieberman L, Greinacher A, Murphy MF, Bussel J, Bakchoul T, Corke S, et al. Fetal and neonatal alloimmune thrombocytopenia: recommendations for evidence-based practice, an international approach. Br J Haematol 2019;185:549–62.

[7] Schmitt A, Guichard J, Massé J-M, Debili N, Cramer EM. Of mice and men: comparison of the ultrastructure of megakaryocytes and platelets. Exp Hematol 2001;29:1295–302.

[8] Barrios M, Rodríguez–Acosta A, Gil A, Salazar AM, Taylor P, Sánchez EE, et al. Comparative hemostatic parameters in BALB/c, C57BL/6 and C3H/He mice. Thromb Res 2009;124:338–43.

[9] Liu Z-J, Hoffmeister KM, Hu Z, Mager DE, Ait-Oudhia S, Debrincat MA, et al. Expansion of the neonatal platelet mass is achieved via an extension of platelet lifespan. Blood 2014;123:3381–9.

[10] Aurbach K, Spindler M, Haining EJ, Bender M, Pleines I. Blood collection, platelet isolation and measurement of platelet count and size in mice—a practical guide. Platelets 2019;30:698–707.

[11] Fukuda T, Asou E, Nogi K, Goto K. Evaluation of mouse red blood cell and platelet counting with an automated hematology analyzer. J Vet Med Sci 2017;79:1707–11.

[12] Mouse Phenotype Database RRID:SCR_003212 [Internet] Bar Harbor (ME). The Jackson Laboratory. C2004 - [cited 2019 Nov 11]. Available from: https://phenome.jax.org/.

[13] Cieutat AM, Rosa JP, Letourneur F, Poncz M, Rifat S. A comparative analysis of cDNA-derived sequences for rat and mouse β3 integrins (GPIIIA) with their human counterpart. Biochem Biophys Res Commun 1993;193:771–8.

[14] Smyth SS, Tsakiris DA, Scudder LE, Coller BS. Structure and function of murine αIIbβ3 (GPIIb/IIIa): studies using monoclonal antibodies and β3-null mice. Thromb Haemost 2000;84:1103–8.

[15] Barron-Casella EA, Nebbia G, Rogers OC, King KE, Kickler TS, Casella JF. Construction of a human platelet alloantigen-1a epitope(s) within murine glycoprotein IIIa: identification of residues critical to the conformation of the antibody binding site(s). Presented in part at the American Society of Hematology meeting, December 5–9, 1997, 93. 1999. p. 2959–67.

[16] Schmidt A, Morales-Prieto DM, Pastuschek J, Fröhlich K, Markert UR. Only humans have human placentas: molecular differences between mice and humans. J Reprod Immunol 2015;108:65–71.

[17] Kim J, Mohanty S, Ganesan LP, Hua K, Jarjoura D, Hayton WL, et al. FcRn in the yolk sac endoderm of mouse is required for IgG transport to fetus. J Immunol 2009;182:2583–9.

[18] Sellers RS, Clifford CB, Treuting PM, Brayton C. Immunological variation between inbred laboratory mouse strains: points to consider in phenotyping genetically immunomodified mice. Vet Pathol 2011;49:32–43.

[19] Mills CD, Kincaid K, Alt JM, Heilman MJ, Hill AM. M-1/M-2 macrophages and the Th1/Th2 paradigm. J Immunol 2000;164:6166–73.

[20] Ni H, Chen P, Spring CM, Sayeh E, Semple JW, Lazarus AH, et al. A novel murine model of fetal and neonatal alloimmune thrombocytopenia: response to intravenous IgG therapy. Blood 2006;107:2976–83.

[21] Hodivala-Dilke KM, McHugh KP, Tsakiris DA, Rayburn H, Crowley D, Ullman-Culleré M, et al. β3-integrin–deficient mice are a model for Glanzmann thrombasthenia showing placental defects and reduced survival. J Clin Invest 1999;103:229–38.

[22] Ware J, Russell S, Ruggeri ZM. Generation and rescue of a murine model of platelet dysfunction: the Bernard-Soulier syndrome. Proc Natl Acad Sci U S A 2000;97:2803–8.

[23] Li C, Piran S, Chen P, Lang S, Zarpellon A, Jin JW, et al. The maternal immune response to fetal platelet GPIbα causes frequent miscarriage in mice that can be prevented by intravenous IgG and anti-FcRn therapies. J Clin Invest 2011;121:4537–47.

[24] Fang J, Hodivala-Dilke K, Johnson BD, Du LM Hynes RO, White 2nd GC, et al. Therapeutic expression of the platelet-specific integrin, alphaIIbbeta3, in a murine model for Glanzmann thrombasthenia. Blood 2005;106:2671–9.

[25] Ghevaert C, Wilcox DA, Fang J, Armour KL, Clark MR, Ouwehand WH, et al. Developing recombinant HPA-1a–specific antibodies with abrogated Fcγ receptor binding for the treatment of fetomaternal alloimmune thrombocytopenia. J Clin Invest 2008;118:2929–38.

[26] Shultz LD, Schweitzer PA, Christianson SW, Gott B, Schweitzer IB, Tennent B, et al. Multiple defects in innate and adaptive immunologic function in NOD/LtSz-scid mice. J Immunol 1995;154:180–91.

[27] Webster ML, Sayeh E, Crow M, Chen P, Nieswandt B, Freedman J, et al. Relative efficacy of intravenous immunoglobulin G in ameliorating thrombocytopenia induced by antiplatelet GPIIbIIIa versus GPIbα antibodies. Blood 2006;108:943–6.

[28] Chen P, Li C, Lang S, Zhu G, Reheman A, Spring CM, et al. Animal model of fetal and neonatal immune thrombocytopenia: role of neonatal Fc receptor in the pathogenesis and therapy. Blood 2010;116:3660–8.

[29] Liu L, Garcia AM, Santoro H, Zhang Y, McDonnell K, Dumont J, et al. Amelioration of experimental autoimmune myasthenia gravis in rats by neonatal FcR blockade. J Immunol 2007;178:5390–8.

[30] Ober RJ, Radu CG, Ghetie V, Ward ES. Differences in promiscuity for antibody–FcRn interactions across species: implications for therapeutic antibodies. Int Immunol 2001;13:1551–9.

[31] Andersen JT, Bekele Daba M, Berntzen G, Michaelsen TE, Sandlie I. Cross-species binding analyses of mouse and human neonatal Fc receptor show dramatic differences in immunoglobulin G and albumin binding. J Biol Chem 2010;285:4826–36.

[32] Sand KMK, Bern M, Nilsen J, Noordzij HT, Sandlie I, Andersen JT. Unraveling the interaction between FcRn and albumin: opportunities for design of albumin-based therapeutics. Front Immunol 2015;5:682.

[33] Roy S, Nanovskaya T, Patrikeeva S, Cochran E, Parge V, Guess J, et al. M281, an anti-FcRn antibody, inhibits IgG transfer in a human ex vivo placental perfusion model. Am J Obstet Gynecol 2019;220(498):e1–9.

[34] Clinical trial. A study to evaluate the safety, efficacy, pharmacokinetics and pharmacodynamics of M281 administered to pregnant women at high risk for early onset severe hemolytic disease of the fetus and newborn (HDFN). 2019https://ClinicalTrials.gov/show/NCT03842189.

[35] Clinical trial. A study to characterize the clinical course of pregnant women and children at high risk for early onset severe hemolytic disease of the fetus and newborn. 2019https://ClinicalTrials.gov/show/NCT03755128.

[36] Bakchoul T, Boylan B, Sachs UJH, Bein G, Ruan C, Santoso S, et al. Blockade of maternal anti-HPA-1a–mediated platelet clearance by an HPA-1a epitope–specific F(ab′)2 in an in vivo mouse model of alloimmune thrombocytopenia. Transfusion 2009;49:265–70.

[37] Boylan B, Berndt MC, Kahn ML, Newman PJ. Activation-independent, antibody-mediated removal of GPVI from circulating human platelets: development of a novel NOD/SCID mouse model to evaluate the in vivo effectiveness of anti–human platelet agents. Blood 2006;108:908–14.

[38] Bakchoul T, Greinacher A, Sachs UJ, Krautwurst A, Renz H, Harb H, et al. Inhibition of HPA-1a alloantibody-mediated platelet destruction by a deglycosylated anti–HPA-1a monoclonal antibody in mice: toward targeted treatment of fetal-alloimmune thrombocytopenia. Blood 2013;122:321–7.

[39] Jefferis R. Glycosylation as a strategy to improve antibody-based therapeutics. Nat Rev Drug Discov 2009;8:226–34.

[40] Ahlen MT, Husebekk A, Killie MK, Skogen B, Stuge TB. T-cell responses associated with neonatal alloimmune thrombocytopenia: isolation of HPA-1a-specific, HLA-DRB3*0101-restricted CD4+ T cells. Blood 2009;113:3838–44.

[41] Parry CS, Gorski J, Stern LJ. Crystallographic structure of the human leukocyte antigen DRA, DRB3*0101: models of a directional alloimmune response and autoimmunity. J Mol Biol 2007;371:435–46.

[42] Rayment R, Kooij TW, Zhang W, Siebold C, Murphy MF, Allen D, et al. Evidence for the specificity for platelet HPA-1a alloepitope and the presenting HLA-DR52a of diverse antigen-specific helper T cell clones from alloimmunized mothers. J Immunol 2009;183:677–86.

[43] Wu S, Maslanka K, Gorski J. An integrin polymorphism that defines reactivity with alloantibodies generates an anchor for MHC class II peptide binding: a model for unidirectional alloimmune responses. J Immun 1997;158:3221–6.

[44] Sukati H, Bessos H, Barker RN, Urbaniak SJ. Characterization of the alloreactive helper T-cell response to the platelet membrane glycoprotein IIIa (integrin-β3) in human platelet antigen-1a alloimmunized human platelet antigen-1b1b women. Transfusion 2005;45:1165–77.

[45] Jackson DJ, Murphy MF, Soothill PW, Lucas GF, Elson CJ, Kumpel BM. Reactivity of T cells from women with antibodies to the human platelet antigen (HPA)-1a to peptides encompassing the HPA-1 polymorphism. Clin Exp Immunol 2005;142:92–102.

[46] J. Kjeldsen-Kragh, M. Ahlen, Foetal and Neonatal Alloimmune Thrombocytopenia – The role of the HLA-DRB3*01:01 allele for HPA-1a-immunisation and foetal/neonatal outcome. Transfusion and Apheresis Science. XXXX;XX:XX-XX https://doi.org/10.1016/j.transci.2019.102707.
[47] Jackson DJ, Eastlake JL, Kumpel BM. Human platelet antigen (HPA)-1a peptides do not reliably suppress anti-HPA-1a responses using a humanized severe combined immunodeficiency (SCID) mouse model. Clin Exp Immunol 2014;176:23–36.
[48] Wraith DC. Designing antigens for the prevention and treatment of autoimmune diseases. Curr Opin Chem Eng 2018;19:35–42.
[49] Killie MK, Husebekk A, Kjeldsen-Kragh J, Skogen B. A prospective study of maternal anti-HPA 1a antibody level as a potential predictor of alloimmune thrombocytopenia in the newborn. Haematologica 2008;93:870–7.
[50] Kumpel BM, Elson CJ. Mechanism of anti-D-mediated immune suppression – a paradox awaiting resolution? Trends Immunol 2001;22:26–31.
[51] Marjoram D, Cruz-Leal Y, Bernardo L, Lazarus AH. A role for red cell clearance in antibody-mediated inhibition of erythrocyte alloimmunization? ISBT Sci Ser 2017;12:196–201.
[52] Bernardo L, Yu H, Amash A, Zimring JC, Lazarus AH. IgG-mediated immune suppression to erythrocytes by polyclonal antibodies can occur in the absence of activating or inhibitory Fcγ receptors in a full mouse model. J Immunol 2015;195:2224–30.
[53] Tiller H, Killie MK, Chen P, Eksteen M, Husebekk A, Skogen B, et al. Toward a prophylaxis against fetal and neonatal alloimmune thrombocytopenia: induction of antibody-mediated immune suppression and prevention of severe clinical complications in a murine model. Transfusion 2012;52:1446–57.
[54] Li C, Chen P, Vadasz B, Ma L, Zhou H, Lang S, et al. Co-stimulation with LPS or poly I:C markedly enhances the anti-platelet immune response and severity of fetal and neonatal alloimmune thrombocytopenia. Thromb Haemost 2013;110:1250–8.
[55] Hendrickson JE, Desmarets M, Deshpande SS, Chadwick TE, Hillyer CD, Roback JD, et al. Recipient inflammation affects the frequency and magnitude of immunization to transfused red blood cells. Transfusion 2006;46:1526–36.
[56] Elayeb R, Tamagne M, Bierling P, Noizat-Pirenne F, Vingert B. Red blood cell alloimmunization is influenced by the delay between Toll-like receptor agonist injection and transfusion. Haematologica 2016;101:209–18.
[57] Yougbaré I, Lang S, Yang H, Chen P, Zhao X, Tai W-S, et al. Maternal anti-platelet β3 integrins impair angiogenesis and cause intracranial hemorrhage. J Clin Invest 2015;125:1545–56.
[58] Santoso S, Wihadmadyatami H, Bakchoul T, Werth S, Al-Fakhri N, Bein G, et al. Antiendothelial αvβ3 antibodies are a major cause of intracranial bleeding in fetal/neonatal alloimmune thrombocytopenia. Arterioscler Thromb Vasc Biol 2016:1517–24.
[59] Yougbaré I, Tai W-S, Zdravic D, Oswald BE, Lang S, Zhu G, et al. Activated NK cells cause placental dysfunction and miscarriages in fetal alloimmune thrombocytopenia. Nat Commun 2017;8:224.
[60] Bakchoul T, Kubiak S, Krautwurst A, Roderfeld M, Siebert HC, Bein G, et al. Low-avidity anti-HPA-1a alloantibodies are capable of antigen-positive platelet destruction in the NOD/SCID mouse model of alloimmune thrombocytopenia. Transfusion 2011;51:2455–61.
[61] Peterson JA, Kanack A, Nayak D, Bougie DW, McFarland JG, Curtis BR, et al. Prevalence and clinical significance of low-avidity HPA-1a antibodies in women exposed to HPA-1a during pregnancy. Transfusion 2013;53:1309–18.
[62] Kjeldsen-Kragh J, Titze TL, Lie BA, Vaage JT, Kjær M. HLA-DRB3*01:01 exhibits a dose-dependent impact on HPA-1a antibody levels in HPA-1a–immunized women. Blood Adv 2019;3:945–51.
[63] Xiao T, Takagi J, Coller BS, Wang J-H, Springer TA. Structural basis for allostery in integrins and binding to fibrinogen-mimetic therapeutics. Nature 2004;432:59–67.
[64] Thinn AMM, Wang Z, Zhou D, Zhao Y, Curtis BR, Zhu J. Autonomous conformational regulation of β_3 integrin and the conformation-dependent property of HPA-1a alloantibodies. Proc Natl Acad Sci U S A 2018;115:E9105–14.
[65] Valentin N, Visentin G, Newman P. Involvement of the cysteine-rich domain of glycoprotein IIIa in the expression of the human platelet alloantigen, PlA1: evidence for heterogeneity in the humoral response. Blood 1995;85:3028–33.
[66] Zhi H, Ahlen MT, Thinn AMM, Weiler H, Curtis BR, Skogen B, et al. High-resolution mapping of the polyclonal immune response to the human platelet alloantigen HPA-1a (Pl^{A1}). Blood Adv 2018;2:3001.