Maternal microchimerism protects hemophilia A patients from inhibitor development

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Key Points

- Deleterious F8 mutations do not necessarily lead to the incidence of inhibitors in hemophilia A patients receiving replacement therapy.
- Maternal chimeric cells migrated into a fetus with hemophilia A during pregnancy could induce tolerance toward FVIII.

Introduction

Supplementation of plasma-derived or recombinant factor VIII (FVIII) is the mainstay treatment of hemophilia A; however, an alloantibody against exogenous FVIII develops soon after initiation of this treatment regimen in some patients, and renders replacement therapy ineffective.1 Patients with moderate and mild hemophilia A are less likely to be affected by inhibitors, and patients with severe hemophilia A caused by missense mutation may also be spared from alloimmunity against administered FVIII, possibly as a result of the expression of a FVIII variant with just a single amino acid residue substitution, albeit mostly at low level. The deleterious F8 mutations, such as large deletions/insertions and intron 22 inversion, lead to null expression of FVIII and tend to pose a higher risk for the incidence of inhibitors.2,3 However, the development of alloantibody cannot be exclusively attributed to the absence of endogenous FVIII: with only 30% to 40% of patients carrying the same deleterious mutations affected by inhibitors,4,5 genetic mutation types and the absence of FVIII protein in circulation alone are not only the factors associated with inhibitor development when patients are exposed to exogenous FVIII in replacement therapy.

The placenta separates the fetus from the mother, but it does not completely block bidirectional trafficking of fetal and maternal cells.6,7 The maternal cells cross the placenta and colonize in large numbers in fetuses with severe combined immunodeficiency lacking a defensive immune system, and cells of maternal origin can also be found in immune-competent infants at a much lower level.6 Maternally derived hematopoietic stem cells are able to differentiate into cells of erythroid, lymphoid, and myeloid lineages, including antigen-presenting macrophages and dendritic cells.8 Fetal exposure to maternal noninherited antigens in utero induces stable immune tolerance to accommodate genetically discordant maternal tissue.9,10 It has been suggested that T cells arose from hematopoietic stem cells at different stages of development and are distinct populations, with fetal T-cell lineage biased toward immune tolerance. Maternal cells induce the development of CD4+CD25highFoxP3+ Tregs in fetal lymph nodes, which suppress fetal immunity against maternal antigen and persist at least until early adulthood, with both physiological and pathological implications.11-13

Recent studies revealed that FVIII originates primarily from liver sinusoidal endothelial cells, and peripheral blood cells, particularly monocytes, may also be one of the extrahepatic sources of FVIII in circulation.14,15 It has been shown that bone marrow cells modified with human FVIII transplanted to hemophilic mice could induce immune tolerance toward FVIII by the induction of antigen-specific regulatory T cells.16,17 Thus, the presence of maternal chimeric cells in patients with hemophilia A (HA) capable of differentiating into leukocytes and synthesizing FVIII raises the interesting question of whether FVIII-bearing maternal cells could also be a source for tolerance induction toward FVIII during maturation of the fetal immune system in pregnancy. We thus conducted a cross-sectional study to explore the implications of maternal micromicrochimerism for the development of inhibitors in patients with hemophilia A.

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Data sharing is available by sending an e-mail to the corresponding author, Wenman Wu (wenmanwu@shsmu.edu.cn).

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Methods

Study subjects

We surveyed 117 unrelated patients with severe HA (with FVIII:C <1%) caused by the high inhibitor risk type of mutations, including intron 22, intron 1 inversions, large deletions/insertions, and frame shift and nonsense mutations. All the patients investigated in the current study were placed on plasma-derived factor; only a few had used recombinant FVIII occasionally, and none of them were treated by recombinant FVIII alone. Almost all patients received prophylactic therapy, but they were also given FVIII agents on demand on hemostatic challenges (Table 1). Among the patients investigated, 30 had developed inhibitor levels higher than 5 BU (range, 5.9–143 BU), using the Nijmegen-Bethesda assay (Table 1). The study was approved by the institutional review boards of the Beijing Children’s Hospital of Capital Medical University of China, with informed consent obtained from the parents of the patients.

Maternal microchimerism detection

Three single nucleotide polymorphism (SNP) loci (rs2885051, rs11260221, and rs5910801) on different regions of the X chromosome with high minor allele frequency in Chinese people were selected as markers for individual DNA identification and screened by quantitative polymerase chain reaction (PCR) in the mothers of the patients. Eighty-three mothers were heterozygous for at least 1 of the SNP loci to be heterozygous for maternal-only SNP alleles to some degree (average, 2.8/10 000; range, 1.36/10 000–8.64/10 000). Among the maternal microchimerism-positive patients, only 2 had inhibitors present (10.0%, 2/20), whereas 36.5% of maternal microchimerism-negative patients had inhibitors (23/63). The survey of the current study on the status of maternal chimeras and FVIII inhibitor incidence is consistent with the hypothesis that patients with their maternal genomic material detected by ddPCR are less likely to be associated with inhibitors. The odds ratio of the absence of detectable maternal microchimerism and development of inhibitors is only 5.18 (95% confidence interval, 1.10–24.34; P = .0374) (Table 2), which does not change significantly (odds ratio, 4.92; 95% confidence interval, 1.04–23.28) after the adjustment of patients’ age.

Statistical analysis

The statistical analyses were performed using SAS v. 9.2 (SAS Institute Inc.) to probe the relationship between maternal microchimerism and inhibitor development. The continuous variables were expressed as median (range) and were analyzed using the Mann-Whitney U test. The categorical data were presented as frequencies and percentages and were analyzed by χ² test or Fisher’s exact test. A univariate logistic regression model was used to predict the presence of inhibitors to FVIII, with status of maternal microchimerism being an independent variable, and then a multivariate logistic regression model was fitted for the effect of maternal microchimerism with age as covariate. P < .05 was considered statistically significant.

Results and discussion

Eighty-three of 117 mothers were informative for the microchimerism analysis. Twenty patients with hemophilia A were found to be positive for maternal chimerism, as determined by ddPCR, with at least 1 of the SNP loci to be heterozygous for maternal-only SNP alleles to some degree (average, 2.8/10 000; range, 1.36/10 000–8.64/10 000). Among the maternal microchimerism-positive patients, only 2 had inhibitors present (10.0%, 2/20), whereas 36.5% of maternal microchimerism-negative patients had inhibitors (23/63). The survey of the current study on the status of maternal chimeras and FVIII inhibitor incidence is consistent with the hypothesis that patients with their maternal genomic material detected by ddPCR are less likely to be associated with inhibitors. The odds ratio of the absence of detectable maternal microchimerism and development of inhibitors is only 5.18 (95% confidence interval, 1.10–24.34; P = .0374; Table 2), which does not change significantly (odds ratio, 4.92; 95% confidence interval, 1.04–23.28) after the adjustment of patients’ age.

Most patients’ mothers are carriers of the F8 mutation, and 2 X chromosomes of maternal chimeric hematopoietic cells are subjected to random inactivation just like all the other cells of the mothers.20 Because of the small number of maternal cells that exist in the patients, the skewed inactivation of 1 of the X chromosomes potentially leads to significant variance of FVIII expressed and tolerance induction, which may account for the development of inhibitors in patients even when maternal cells are detected.

In conclusion, we found that maternal microchimerism is an important factor in FVIII immune tolerance, and a high degree of maternal cell mosaicism provides protection against inhibitor development, which may provide a clue for assessment of the risk for inhibitor development.

Table 1. Characteristics of the patients surveyed for maternal microchimerism

| Characteristic | Developed inhibitor (N = 25) | Without inhibitor (N = 58) |
|---------------|-----------------------------|--------------------------|
| Age, y        |                             |                          |
| Range         | 0.6–21                      | 0.6–13                   |
| Average       | 5.53                        | 6.53                     |
| Type of mutation, n (%) |                  |                          |
| Intron 22 inversion | 10 (40)                    | 53 (91)                  |
| Intron 1 inversion | 0 (0)                      | 1 (1.8)                  |
| Nonsense/frameshift | 12 (48)                    | 4 (7.2)                  |
| Large deletion | 3 (12)                      | 0 (0)                    |

Table 2. Odds ratios of inhibitors to FVIII development according to status of maternal microchimerism status among 83 Chinese patients with hemophilia A

| Status of microchimerism | Without inhibitor | With inhibitor | Odds ratio (95% confidence interval) |
|--------------------------|-------------------|----------------|-------------------------------------|
| Microchimerism-negative  | 40                | 23             | Reference group                      |
| Microchimerism-positive  | 18                | 2              | 5.175 (1.100–24.34; P = .0374)        |
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Authorship

Contribution: Y.L. and X.W. performed digital PCR experiments; Y.L., Z.C., and W.W. drafted the manuscript; Z.C. and R.W. recruited subjects in the study; Z.C., J.D., H.G., Z.L., and Q.D. conducted genetic analysis of F8 mutations and coagulation functions; Z.C., H.G., and Z.L. collected samples; J.L. and W.W. contributed to statistical analysis and interpretation; W.W. conceived the project; W.W., R.W., and X.W. designed the study; and all authors edited and approved the manuscript.

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