PLATELETS AND THROMBOPOIESIS

Persistent neonatal thrombocytopenia can be caused by IgA antiplatelet antibodies in breast milk of immune thrombocytopenic mothers

Hagit Hauschner,[1] Nurit Rosenberg,[1] Uri Seligsohn,[1] Rafael Mendelsohn,[2,3] Aryeh Simmonds,[3,4] Yakov Shiff,[3,4] Yaakov Schachter,[2,3] Shraga Aviner,[5] and Nechama Sharon[3,6]

[1]Amalia Biron Research Institute of Thrombosis and Hemostasis, Chaim Sheba Medical Center, Tel Hashomer and Sackler Faculty of Medicine, Tel-Aviv University, Tel-Aviv, Israel; [2]Pediatric Department, Tel Aviv Sourasky Medical Center, Tel Aviv, Israel; [3]Ruth and Bruce Rappoport Faculty of Medicine, Technion, Haifa, Israel; [4]Neonatology Unit, Laniado Hospital, Netanya, Israel; [5]Department of Pediatrics, Barzilai University Medical Center, Ashkelon, and Faculty of Health Sciences, Ben-Gurion University of the Negev, Beer Sheva, Israel; and [6]Pediatric Hemato-oncology Department, Laniado Hospital, Netanya, Israel

Key Points

- Persistent thrombocytopenia was observed in breastfed neonates of ITP women.
- Breast milk of ITP women may contain immunoglobulin A antiplatelet antibodies, which target $\alpha_{IIb}\beta_3$ integrin.

Immune thrombocytopenia (ITP) in pregnant women can cause neonatal thrombocytopenia by transport of antiplatelet autoantibodies across the placenta. Usually, an infant's platelet count normalizes within 2 months. We observed neonatal thrombocytopenia that persisted more than 4 months and disappeared following discontinuation of breastfeeding. The aim of our study was to discern whether breast milk of ITP mothers contained antiplatelet antibodies causing persistent thrombocytopenia. We collected milk samples from 3 groups of women: ITP group, 7 women who had ITP during pregnancy; R-ITP group, 6 women who recovered from ITP before pregnancy; and 9 healthy controls. We found increased levels of antiplatelet antibodies of the immunoglobulin A type in the milk of ITP patients compared with the other 2 groups. Similar increase was demonstrated for antibodies binding to $\alpha_{IIb}\beta_3$ expressed in cultured cells. Thus, transfer of antiplatelet antibodies from ITP mothers by breastfeeding can be associated with persistent neonatal thrombocytopenia. (*Blood*. 2015;126(5):661-664)

Introduction

Immune thrombocytopenia (ITP) is present in 4.1% of thrombocytopenic pregnant women.[1] In ITP patients, immunoglobulin G (IgG) autoantibodies can be formed, targeting platelet $\alpha_{IIb}\beta_3$ or glycoprotein Ib-IX. Maternal IgG can be actively transported across the placenta resulting in neonatal thrombocytopenia. Following decline in neonatal platelet count during the 7 days after delivery, the count gradually increases corresponding to the half-life of the passively transported maternal antiplatelet IgG. Usually, normalization of the infant's platelet count occurs within 2 months.[2]

We recently observed a neonate of a mother with ITP whose platelet count was persistently low for 4 months and recovered upon discontinuation of breastfeeding. The objective of our study was to discern the mechanism responsible for the persistently low platelet count in breastfed newborns and examine additional thrombocytopenic neonates whose mothers had ITP during pregnancy. Another group of patients were women who had a history of ITP yet had normal platelet counts during the current pregnancy and no neonatal ITP.

Study design

Index patient

A.H. (age 36 years) had severe ITP during pregnancy and was treated with corticosteroids and intravenous gamma globulin (IVGG) infusions. At term, her platelet count was 16 000/μL and her newborn's count was 42 000/μL. The infant's counts reached a nadir of 25 000/μL at 1 month. At 3 months, breastfeeding was discontinued, and within 1 month the platelet count recovered. A milk sample was taken 1 month after delivery.

Groups of patients

Milk samples were obtained from 6 additional ITP patients whose neonates had thrombocytopenia (Table 1). Maternal ITP was diagnosed by exclusion of other causes of thrombocytopenia, presence of purpura, and no antiphospholipid antibodies.

Six other patients (designated R-ITP) had a history of ITP but recovered before their current pregnancy. Normal platelet counts were observed in these women and their neonates. Milk was also obtained from 9 healthy women. The study was approved by the institutional review boards of Laniado Hospital and Tel Aviv Sourasky Medical Center and conducted in accordance with the Declaration of Helsinki.

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Table 1. Clinical and laboratory features of ITP and R-ITP lactating patients

	Mother's clinical data			Infant's clinical data				Milk-Ig antibodies		
								Antiplatelet*†		Anti-$\alpha_{IIb}\beta_3$*‡
Group and patient	ITP diagnosis	Treatment during pregnancy	Platelet count on delivery day	Platelet count on delivery day (Nadir)	Treatment	Approximate lactating period (months)§	Stable normal count (months)	IgA/IgM/IgG	IgA	IgA/IgM/IgG
ITP										
A.H.	During pregnancy	Corticosteroids and IVGG	16 000	42 000 (22 000)‖	None	3	4.5	3/3	3/3	5/6
A.L.	During pregnancy	Corticosteroids	56 000	29 000 (17 000)‖	Corticosteroids and IVGG	4	4	6/6	2/3	2/3
S.H.	Diagnosed 10 y ago; splenectomized	Corticosteroids	100 000	8000 (4000)‖	Corticosteroids and IVGG	2.5	3.5	0/3	3/3	2/3
S.I.	3 y before pregnancy	None	60 000	20 000 (20 000)‖	Corticosteroids and IVGG	3	3.5	3/6	0/4	2/5
R.R.	Diagnosed at age 17 y, splenectomized	Corticosteroids	79 000	62 000 (62 000)	None	>6	0.3	3/6	1/3	2/3
R.Be.	During pregnancy	None	49 000	43 000 (15 000)‖	IVGG	2	3.5	3/7	3/7	2/5
R.A.H.	During pregnancy	Steroids and IVGG	36 000	48 000 (15 000)‖	Corticosteroids and IVGG	8	2	1/3	2/3	1/3
R-ITP										
M.A.	Splenectomy	None	200 000	150 000	NR	2	NR	3/4	0/4	2/2
B.K.	Thrombocytopenia in pregnancies	None	118 000	235 000	NR	10	NR	2/2	0/3	1/4
R.Bl.	ITP at age 16 y	None	100 000	260 000	NR	3	NR	2/4	0/3	0/2
A.G.	ITP at age 16 y	None	303 000	240 000	NR	2	NR	1/3	3/3	0/4
L.S.	ITP at age 16 y	None	177 000	124 000	NR	2	NR	1/3	3/3	0/4
H.N.	Thrombocytopenia in pregnancies	None	90 000	133 000	NR	2	NR	1/3	0/3	0/2

Milk-Ig, total Ig extract from breast milk; NR, not relevant.
*Frequency of positive results: the number of positive assays divided by total experiments preformed with the sample (using different platelet donors)
†Washed platelet assay.
‡$\alpha_{IIb}\beta$ 3 cell assay.
§Lactating period is according to mothers' statement. Toward the end of the period, formula was also used in parallel.
‖Full details of the clinical data and treatment are provided in supplemental Figure 1.

Detection of antiplatelet antibodies by using washed platelets

Platelets from 3 healthy donors were prepared by centrifugation and washing and were then mixed together. The washed platelets were incubated with Ig extracted from the milk by using Ig Adem Kit (Odemtech, Pessac, France). Details are provided in supplemental Data available at the *Blood* Web site. Antiplatelet antibodies were detected by anti-human total Ig (IgA/IgM/IgG)-phycoerythrin (Millipore, Temecula, CA), anti-human IgG-fluorescein isothiocyanate (Dako, Glostrup, Denmark), or anti-human IgA-fluorescein isothiocyanate (Millipore). The samples were tested by flow cytometry. Samples were considered positive when the median fluorescence intensity (MFI) was greater than the mean MFI plus 2 standard deviations of control samples. Each sample was tested at least 3 times using different platelet donors. The frequency of positive assays for each sample was calculated by determining the ratio between positive results and total experiments performed with the sample. Statistical analyses were performed by comparing the frequencies of detected antibody in ITP women to the R-ITP group or controls with GraphPad software using 2-way analysis of variance with Tukey's multiple comparisons test.

Detection of anti-$\alpha_{IIb}\beta_3$ antibodies by using cultured cells

Anti-$\alpha_{IIb}\beta_3$ antibodies were detected by using baby hamster kidney cells expressing $\alpha_{IIb}\beta_3$ or only the vectors (mock). Expression of $\alpha_{IIb}\beta_3$ on the cell surface was validated by using monoclonal antibodies against $\alpha_{IIb}\beta_3$3 (supplemental Data). The cell suspension in phosphate-buffered saline was incubated with milk-Ig samples for 30 minutes. Anti-$\alpha_{IIb}\beta_{33}$ antibodies were detected by adding total anti-human Ig-phycoerythrin (Millipore) to the cells and using flow cytometry. The ratio of anti-$\alpha_{IIb}\beta_3$ antibody binding was calculated by dividing the MFI of $\alpha_{IIb}\beta_3$-expressing cells by the MFI of mock cells incubated with the same milk-Ig sample. Samples were considered positive when the results were greater than mean ratios of control samples plus 2 standard deviations.

Results and discussion

Representative patient

A.H. had ITP diagnosed during pregnancy, and her infant had thrombocytopenia that persisted for 4 months. We examined A.H.'s milk sample for antiplatelet antibodies by using an antibody that recognizes IgG, IgA, and IgM. Figure 1A demonstrates a high level of antiplatelet antibodies in the milk-Ig sample from A.H. compared with a milk-Ig sample from a control. Next, we tested whether the antibodies were of IgG, as in the sera of most patients with chronic ITP, or IgA, the predominant type in breast milk. The milk sample from A.H. contained antiplatelet antibodies that were solely IgA (Figure 1B-C). To identify the specific antigen against which the antibodies reacted, we tested the Ig samples with cultured cells expressing integrin $\alpha_{IIb}\beta_3$ and compared the results to mock cells. Figure 1D shows that anti-$\alpha_{IIb}\beta_3$ antibodies were abundant in A.H.'s milk-Ig sample.

Presence of antiplatelet antibodies in the milk of the 3 groups of patients

Table 1 lists clinical and immunologic data for 7 ITP and 6 R-ITP patients and their neonates. ITP patients had ITP during pregnancy, and their neonates were born with thrombocytopenia that lasted more than

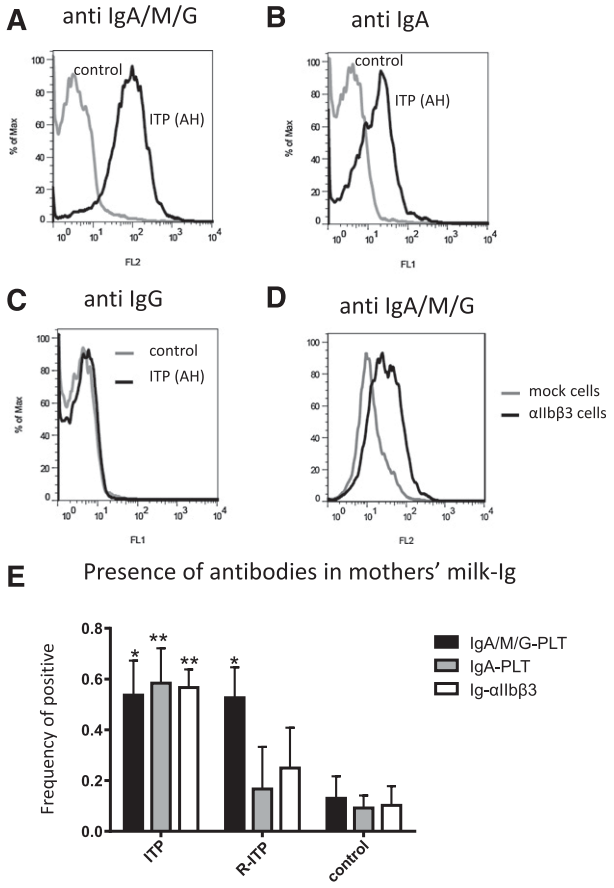

Figure 1. Antiplatelet antibodies in Ig extracts of breast milk. (A-C) Flow cytometry histograms of antiplatelet antibodies in milk-Ig of A.H. (a representative ITP patient [black line]) and controls (gray line) detected by anti-human antibodies: IgA/IgM/IgG-phycoerythrin, anti-IgA-fluorescein isothiocyanate, and anti-IgG-fluorescein isothiocyanate. (D) Detection of anti-$\alpha_{IIb}\beta_3$ antibodies in milk-Ig from A.H. The milk-Ig was incubated with baby hamster kidney cells expressing $\alpha_{IIb}\beta_3$ (black line) or mock cells (gray line). Antibodies were detected by anti-human IgA/IgM/IgG-phycoerythrin detected in FL2 by flow cytometry. FL1, fluorescence of fluorescein isothiocyanate; FL2, fluorescence of phycoerythrin. (E) Statistical analysis of the 3 different groups of women: ITP patients, R-ITP patients (women who had recovered from ITP and during pregnancy had normal platelet count), and controls. Each column represents mean + standard error of the mean of the frequency of positive results. Frequency was calculated for each sample as the number of positive results among the total number of experiments performed. Analyses were performed by comparing frequencies of detected antibody in ITP and R-ITP women with the controls by using GraphPad software and 2-way analysis of variance with Tukey's multiple comparisons test. $^*P < .05$; $^{**}P < .01$.

3 months in 5 neonates. Five neonates were treated with IVGG and 4 of them also received corticosteroids. There was no response to IVGG according to the standard response criteria for ITP,[4] and corticosteroid dependence was observed in 3 patients (supplemental Figure 1). This suggests that the antiplatelet antibodies were not only residues from IgG placental transfer. Two of ITP women (A.H. and A.L.) exhibited a high level of antiplatelet IgA antibodies as well as anti-$\alpha_{IIb}\beta_3$ in their milk samples. In 3 other patients (S.I., R.R., and S.H.), low levels of antibodies against platelets were found. Altogether, milk samples from 4 of 7 patients exhibited antibodies against $\alpha_{IIb}\beta_3$. These results are significantly different from the results in women who had recovered from ITP and, in the current pregnancy, had normal platelet count as did their neonates (R-ITP patients in Table 1). The recovery was achieved by splenectomy (M.A.) or by treatment with corticosteroids during childhood (R.Bl., L.S., and A.G., data not shown). Two remaining patients (B.K. and H.N.) suffered from mild thrombocytopenia only during previous pregnancies. R-ITP women exhibited a high frequency of positive results for IgA/IgM/IgG compared with controls ($P < .05$) but low frequency of IgA platelet antibodies in their milk compared with ITP women ($P < .05$) (Figure 1E). The milk-Ig was against $\alpha_{IIb}\beta_3$ in only 1 R-ITP woman (M.A.). In controls, 1 of 9 samples was positive for anti-$\alpha_{IIb}\beta_3$ antibody, resulting in a significant difference between the ITP group and controls ($P < .01$).

Our study provides the first evidence for the presence of antiplatelet antibodies in breast milk of ITP patients associated with neonatal persistent thrombocytopenia. These antibodies were of the IgA type and were recognized by epitopes on $\alpha_{IIb}\beta_3$. IgA antibodies can be absorbed along the infant's gastrointestinal tract and gain access to the circulation.[5] Anti-$\alpha_{IIb}\beta_3$ antibody was also found in 1 control sample, which might be explained by the broad spectrum of IgA antibodies found in colostrum samples of healthy mothers, and was suggested to be important for the anti-idiotype network.[6]

The primary mechanism for platelet destruction in ITP is thought to be autoantibody-dependent phagocytosis, especially in cases of anti-$\alpha_{IIb}\beta_3$ antibodies.[2,7] IgA, which interacts with FcαRI expressed on monocytes and macrophages, can initiate antibody-dependent cell-mediated cytotoxicity. Conversely, an infant's immune system is immature during the first few months after birth. Therefore, the mechanism for the breastfed neonate's thrombocytopenia is an issue for further investigation.

The hypothesis that milk from ITP women can cause thrombocytopenia in the infant was discussed in a series of articles and letters. Kelemen et al[8] suggested that the colostrum of ITP patients contributed to the lowering of circulating platelet counts in the first postnatal days. This was supported by high levels of Ig found in colostrum-fed infants on postpartum day 5 compared with formula-fed infants.[9] Hence, lactation was discouraged.[10] In contrast, Meschengieser and Lazzari[11] described an ITP patient who gave birth to a thrombocytopenic preterm infant who was breastfed from day 5 without apparent adverse effects on his platelet count. In his review, Bussel[12] mentioned a patient with persistent thrombocytopenia who recovered only when breastfeeding was avoided. In our view, breastfeeding should not be discouraged, but when low platelet counts persist, discontinuing breastfeeding is a viable solution.

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Authorship

Contribution: H.H. designed and performed the study, interpreted the findings, and wrote the manuscript; N.R. and U.S. designed the study, interpreted the data, and wrote the manuscript; R.M., Y. Shiff, and S.A. identified patients; A.S. and Y. Schachter contributed to writing the article; and N.S. made the initial observation, collected the data, and wrote the article.

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Correspondence: Hagit Hauschner, Amalia Biron Research Institute of Thrombosis and Hemostasis, Chaim Sheba Medical Center, Tel Hashomer, Israel 52621; e-mail: hagit.hauschner@sheba.health.gov.il.

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