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IVIg therapy increases delivery birthweight in babies born to women with elevated preconception proportion of peripheral blood (CD56+/CD3-) natural killer cells

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Summary

Summary: In this study, the authors investigated: (1) whether elevated preconception peripheral blood proportion of $CD56^+/CD3^-$ lymphocytes (NK cells) was associated with low delivery birthweight in high risk women, and (2) whether intravenous immunoglobulin (IVIg) therapy could be used to improve the delivery outcome in these women. *Materials and Methods:* Sixty-six women who had singleton deliveries were divided into four groups. Group 1: 16 women with elevated preconception NK cells (>12%) using IVIg, group 2: eight women with similar elevated preconception NK cells not using IVIg, group 3: 32 women with non-elevated preconception NK cells (≤ 12%) using IVIg, and group IV: ten women with similar non-elevated preconception NK cells not using IVIg. These groups were similar with regards to patient age, test results, and history. *Results:* Mean gestational age (±SD) of babies at delivery was 39.3 ± 1.7, 37.4 ± 3.7, 38.5 ± 1.3, and 38.7 ± 1.5 weeks, for groups 1, 2, 3 and 4, respectively. Mean birthweight of babies at delivery was 3,267 ± 373, 2,654 ± 627, 3,129 ± 527, and 3,202 ± 357 grams, respectively. Birthweight was significantly higher for group 1 *vs.* group 2 ($p = 0.006$) but not for groups 1 *vs*. group 3. There was no significant difference between the groups for preeclampsia rate, C-section rate or preterm delivery rate. *Conclusion:* In women with elevated preconception peripheral NK cells, mean birthweight at delivery is low without IVIg therapy (2,654 ± 627 grams) but significantly improved with IVIg therapy (3,267 ± 373 grams). In high risk women without preconception NK cell elevation, mean birthweight at delivery is not further increased with IVIg therapy (3,202 ± 357 grams with IVIg *vs*. 3,129 ± 527 grams without IVIg). IVIg may be a treatment option for women with preconception NK elevation at risk of a low birthweight baby. Preconception immune testing may be a tool for determining which patients will benefit from IVIg therapy. Larger repeat studies are needed for confirmation.

Key Words: IUGR; IVIg; Low birthweight; Natural killer cell; Preeclampsia; Preterm delivery.

Introduction

A low birth weight baby is defined as a live born infant of less than 2,500 grams or below the 10th percentile for gestational age [1]. In the United States approximately 6.1% (231,900) of deliveries meet these criteria [2]. In developing countries, low birth weight is associated with infant mortality in 60 to 80 percent of cases [3]. In cases where low birth weight is caused by intrauterine growth restriction (IUGR), perinatal mortality is increased four to eight times and morbidity is present in 50% of surviving infants [4, 5]. Low birth weight often co-exists with other pregnancy complications such as preeclampsia, preterm delivery, and poor maternal health [6-9].

Many maternal factors associated with low birth weight share an inflammatory component. These comprise a wide range of conditions such as heart disease [10, 11], periodontal disease [12], recurrent miscarriage [13-15], thyroid disease [16], irritable bowel disease [17], and autoimmune disease [18-23]. Abnormalities in a variety of immunological parameters have been suspected of contributing to low birth weight. These include increased ratio of Th1 (TNF-α) to Th2 (IL-10) cytokine-secreting cells [24, 25] activated macrophages [26], altered uterine natural killer (NK) cells [27-29], increased circulating NK cells percentage and activity [30-33].

Intravenous immunoglobulin (IVIg) has been used in the treatment of autoimmune and inflammatory diseases for over 40 years. Diseases approved for treatment with IVIg include Kawasaki disease, dermatomyositis, multiple sclerosis, and graft versus host disease among others [34-37]. Many more disorders are routinely treated with IVIg. Pregnancy failure is one of an extensive list of such off-label indications [38]. In particular, women with a history of recurrent miscarriage associated with elevated preconception peripheral blood NK have been shown to experience higher delivery rates with IVIg therapy (unless otherwise specified, the term "NK cell" shall refer to the proportion of peripheral blood lymphocytes of the CD56+/CD3- phenotype) [39-44].

The present authors conducted a retrospective study to investigate whether IVIg may reduce the incidence of low birthweight in women with a history of recurrent miscarriage and/or infertility and an increase in the proportion of

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peripheral blood NK cells. Clinically, similar patients were selected and segregated into groups based on differences determined by in-vitro assays of immune function. Patients were separated into four groups based upon preconception percentage of peripheral NK cells and use of IVIg. Differences in frequency of multiple parameters for delivery outcome were then assessed and compared.

Materials and Methods

The patients were seen at the Alan E. Beer Center for Reproductive Immunology and Genetics in Los Gatos, CA, USA, a high risk recurrent pregnancy loss and infertility population**.** Testing was performed at the Laboratory for Reproductive Medicine & Immunology in San Jose, California, USA, except for the antiphospholipid antibody (APA) testing and antinuclear antibody (ANA) testing which was sent to outside laboratories. Testing and treatment were performed using standard protocols that target immunologic and coagulation abnormalities.

This was a single-center, retrospective study. All prospective patients had an index cycle between June 2006 and September 2014. All patients selected for the study used anticoagulant therapy during the first trimester of pregnancy. Patients using immunotherapies that target recurrent miscarriage other than IVIg, such as anti-TNFα therapy, intralipid, and lymphocyte immunization Therapy and G-CSF were excluded from the study. Prospective patients were then divided into four groups. Groups 1 and 2 demonstrated a preconception proportion of peripheral blood NK cells > 12%. Groups 3 and 4 exhibited a preconception proportion of NK cells ≤ 12%. Using these combined criteria, 66 eligible deliveries were identified for the study out of roughly 1,000 deliveries available from the allotted time frame. These 66 deliveries were then further divided into four subgroups based on immunologic and therapeutic category: group 1: 16 deliveries with preconception NK cells > 12% being treated with IVIg: group 2: eight deliveries with preconception NK cells > 12% not treated with IVIg, group 3: 32 deliveries with preconception NK cells ≤ 12% using IVIg, and group 4: ten deliveries with preconception NK cells ≤ 12% not using IVIg.

Gestational age at delivery, birthweight, incidence of preeclampsia, and IUGR were then compared between these four groups. Treatment was offered to patients who had one or more of the following test abnormalities: elevated Th1:Th2 (TNFα:IL-10 ratio above 30.3 and/or the IFNγ:IL-10 ratio above 21.0) percentage of NK cells > 12%, NK 50:1 cytotoxicity >15%. Group selection was based on preconception test results not postconception test results. It should be noted that patients in group 3 (preconception NK ≤ 12% using IVIg) may have been offered IVIG in pregnancy if their NK proportion increased to greater than 12% once they became pregnant. Patients in group 4 (those not using IVIg despite elevated preconception NK%) were those women who chose not to undergo IVIg therapy due to current lack of definitive statistical evidence to support its use and cost and/or safety concerns with respect to a blood-derived product. All patients signed consent forms. In addition, patients in groups 1 and 3 signed IVIg consent forms explaining the nature of the medication, the possible risks, and the lack of sufficient proof for evidence-based efficacy.

To reduce the number of confounding variables in the present analysis, the relative equivalency between the four study groups was established for multiple patient parameters. As stated above, all prospective used anticoagulant therapy during the index cycle of conception and the first trimester of pregnancy using similar protocol guidelines. To eliminate factors related to twin and triplet pregnancies, only singleton deliveries were included in this study. In addition, patient medical parameters that may have been correlated with adverse delivery outcome, such as blood sugar level, TSH level, BMI, inherited thrombophilia, autoimmunity (antiphospholipid antibodies, antinuclear antibodies), corticosteroid use, donor egg pregnancy, and history of premature birth were also quantitatively assessed and statistically compared between the four groups (Table 1). All women were of a mature reproductive age (> 28-years-old), were in stable partner relationships, and were actively seeking pregnancy. There were no active smokers, drinkers, recreational drug users or teenage or single mothers included in the patient population. In addition, all patients were being seen at a high risk, self-pay, specialist clinic, and so they likely represent a middle- to upper-class socioeconomic group.

Treatments

Anticoagulant therapy

Because heparin has been shown to have an anti-inflammatory effect and can antagonize coagulation involved with pregnancy loss, all patients included in this study received low molecular weight heparin [45-47]. All four groups of patients included in this study received anticoagulant therapy during the first trimester of pregnancy [48-50]. Patients received 81 mg aspirin daily starting at day one of the cycle of conception through delivery combined with either fondaparinux (2.5 mg daily) or enoxaparin (30 mg twice daily) was started on cycle day 6 of the conception cycle and continued through at least 12 weeks gestational age.

IVIg was administered at 400 mg/kg body weight either during the cycle of conception and/or at least once after a positive pregnancy test with NK cytotoxicity (50:1 effector: target cell killing ratio) of > 15% and/or preconception NK cells > 12%. It should be noted that patients in group 3 (preconception NK ≤ 12%) may have been offered IVIg in pregnancy if the NK proportion increased after conception. Additional IVIg was given if these percentages remained elevated in pregnancy.

Corticosteroids were administered in patients that received a positive antinuclear antibody test result prior to conception or at a positive pregnancy test (note that only preconception ANA results are given in Table 1) [51]. Either prednisone was given five mg twice daily starting on day 6 of the cycle of conception, increased to ten mg twice daily at a positive pregnancy test, tapered then stopped at ten weeks gestation, or dexamethasone was given one mg daily starting on day 6 of the cycle of conception continued at that dose, tapered then stopped at ten weeks gestation.

Patients completed preconception testing an average of 6.2 ± 5.2 months preconception (see Table 1). Samples were drawn at all times during the patient's menstrual cycle as we have found that the cycle day does not significantly affect the peripheral blood NK proportion (laboratory data given in Figure 1).

Flow cytometry was performed on peripheral blood mononuclear cells to assess percentage of NK cells. A percentage of NK cells greater than 12% was defined as elevated [41, 42].

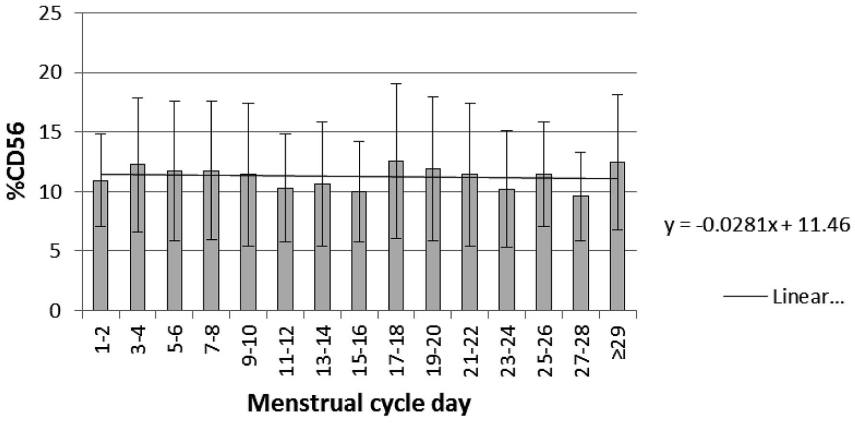
NK cytotoxicity was assessed by flow cytometry, where labeled K562 target cells were incubated with patient mononuclear cells and propidium iodide. Target cell killing was assessed by calculation of the percentage of killed K562 cells identified by dual labelling of target cells at a target to effector cell ratio of 50:1. NK cytotoxicity was tested at an effector to target ratio of 50:1. Cytotoxicity was regarded as elevated when target cell killing ≥ 16 % as we have found it can be associated with an increased rate pregnancy loss [52, 53].

Mitogen stimulation of peripheral blood mononuclear cells was performed in the presence of a cytokine secretion inhibitor (Brefeldin A) followed by membrane permeabilization and combination staining for T helper surface markers and anti-cytokine

Table 1. — *Patient parameters (all four groups used anticoagulant therapy during the first trimester).*

(All test results measured preconception)	Group 1 (NK >12% using IVIg) 16 deliveries	Group 2 (NK >12% not using IVIg) 8 deliveries	Group 3 (NK≤12% using IVIg) 32 deliveries	Group IV (NK≤12% not using IVIg) 10 deliveries	*p* values: Groups 1 + 3 to Groups 2 + 4 (IVIG grps *vs.* no IVIG grps)
Patient history: (mean ± S.D)					
Age (years)	36.9 ± 3.4	39.9 ± 7.8	31.7 ± 4.5	37.6 ± 6.9	NS
Previous live birth	25% (4/16)	25% (2/8)	53% (17/32)	50% (5/10)	NS
Previous premature delivery	13% (2/16)	0% (0/8)	13% (4/32)	40% (4/10)	NS
# prior miscarriages (mean ± SD)	1.2 ± 1.8	1.4 ± 1.8	2.1 ± 1.9	1.3 ± 1.4	NS
Preconception test results:					
# months preconception	7.5 ± 6.3	7.0 ± 5.0	5.9 ± 5.3	4.6 ± 2.8	NS
Clinical testing:					
BMI	23.2 ± 3.9	23.8 ± 6.5	22.9 ± 4.5	23.0 ± 5.4	NS
Glucose (mmol/L)	88.1 ± 6.1	79.6 ± 6.0	79.6 ± 19.4	77.3 ± 21.2	NS
Total insulin (μIU/m)	6.6 ± 3.6	5.0 ± 2.6	6.3 ± 4.5	5.5 ± 3.0	NS
TSH level (mIU/L)	1.9 ± 1.0	1.2 ± 0.8	1.6 ± 0.8	1.3 ± 0.7	NS
Immunological testing:					
TNF-α/IL-10	28.2 ± 5.6	29.4 ± 11.2	26.53 ± 8.1	23.0 ± 3.1	NS
IFN-γ/IL-10	11.2 ± 5.4	12.4 ± 4.3	11.1.0 ± 4.8	13.9 ± 5.4	NS
% CD56+16+	16.7 ± 4.7	17.1 ± 5.8	8.2 ± 5.5	5.4 ± 1.9	NS
% NK 50:1 cytotoxicity	20.7 ± 7.5	16.0 ± 4.0	15.8 ± 8.0	11.5 ± 6.2	NS
% T regulatory cells	0.9 ± 0.4	1.3 ± 1.1	0.9 ± 0.6	1.0 ± 0.3	NS
Pregnancy parameters:					
IVF used	63% (10/16)	88% (7/8)	59% (19/32)	50% (5/10)	NS
Donor egg used	13% (2/16)	25% (2/8)	19% (6/32)	10% (1/10)	NS
+ Inherited thrombophilias	81% (13/16)	88% (7/8)	94% (30/32)	90% (9/10)	NS
Preconception + APA (of pts tested)	38% (6/16)	50% (4/8)	48% (15/31)	40% (4/10)	NS
Preconception + ANA (of pts tested)	19% (3/16)	25% (2/8)	16% (5/31)	20% (2/10)	NS
Corticosteroids used	69%(11/16)	50% (4/8)	63% (20/32)	30% (3/10)	NS
IVIg protocol:					(Groups 1: 3)
Preconception IVIg only	19%(3/16)	NA	16% (5/32)	NA	NS
Preconception and post conception IVIg used	31%(5/16)	NA	25% (8/32)	NA	NS
Postconception IVIg only	50% (8/16)	NA	59% (19/32)	NA	NS
Average no. IVIg doses (mean ± SD)	2.3 ± 1.4	NA	2.3 ± 1.9	NA	NS
Average no. preconception IVIg doses (mean±SD)	0.9±1.0	NA	0.5 ± 0.8	NA	NS
Average no. postconception IVIg doses (mean±SD)	1.4±1.4	NA	1.8 ± 1.8	NA	NS

Figure 1. — Mean peripheal blood CD56 proportion and cycle day in healthy women of reproductive age (512 patient samples).



Mean peripheral blood % CD56 and cycle day

Cycle day	# samples	% peripheral blood NK (CD56)	SD
1-2	22	10.9	3.9
3-4	33	12.2	5.6
5-6	34	11.7	5.9
7-8	30	11.8	5.8
9-10	37	11.4	6.0
11-12	37	10.3	4.5
13-14	50	10.6	5.2
15-16	33	10.0	4.2
17-18	38	12.6	6.5
19-20	48	11.9	6.0
21-22	41	11.4	6.0
23-24	40	10.2	4.9
25-26	27	11.4	4.4
27-28	22	9.6	3.7
≥29	20	12.4	5.7

Table 2. — *Delivery outcomes.*

Study groups: (All test results measured preconception)	Group 1 (NK >12% using IVIg) 16 deliveries	Group 2 (NK >12% not using IVIg) 8 deliveries	Group 3 (NK≤12% using IVIg) 32 deliveries	Group 4 (NK≤12% not using IVIg) 10 deliveries	*p* values:	
Gestational age delivery (weeks)	39.3 ± 1.7	37.4 ± 3.7	38.5 ± 1.3	38.7 ± 1.5	NS	Group 1 to 2
					NS	Group 2 to 4
					= 0.07	Groups 1 to 3
					NS	Groups 3 to 4
					NS	Groups 2 to 3
					NS	Groups 1 to 4
Mean birthweight (grams)	3267 ± 373	2654 ± 627	3129 ± 527	3202 ± 357	= 0.006	Group 1 to 2
					= 0.03	Group 2 to 4
					NS	Groups 1 to 3
					NS	Groups 3 to 4
					= 0.03	Groups 2 to 3
					NS	Groups 1 to 4
% C-section	31% (5/16)	50% (4/8)	48% (15/31)	50% (5/10)	NS	Group 1 to 2
					NS	Group 2 to 4
					NS	Groups 1 to 3
					NS	Groups 3 to 4
					NS	Groups 2 to 3
					NS	Groups 1 to 4
% Preeclampsia	6% (1/16)	25% (2/8)	16% (5/32)	0% (0/10)	NS	Group 1 to 2
					NS	Group 2 to 4
					NS	Groups 1 to 3
					NS	Groups 3 to 4
					NS	Groups 2 to 3
					NS	Groups 1 to 4
% IUGR	6% (1/16)	13% (1/8)	22% (7/32)	10% (1/10)	NS	Group 1 to 2
					NS	Group 2 to 4
					NS	Groups 1 to 3
					NS	Groups 3 to 4
					NS	Groups 2 to 3
					NS	Groups 1 to 4

markers. The Th1/Th2 intracellular cytokine ratio was assessed by measuring the ratio of TNF alpha expressing cells to IL-10 expressing cells (TNFα/IL-10), and interferon gamma expressing cells to IL-10 expressing cells (IFNγ:IL-10). A TNFα:IL-10 ratio above 30.3 and/or the IFNγ:IL-10 ratio above 21.0 were considered elevated as they are associated with decreased fertility rates [24].

T regulatory cells were quantified by flow cytometry following staining for CD4 and CD25 surface membrane-markers and FoxP3+ intracellular marker. Triple positive lymphocytes was reported as T regulatory cells.

IgM, IgG and IgA antibodies directed against six phospholipid antigens (cardiolipin, phosphatidylethanolamine, phosphatidylserine, phosphatidylglycerol, phosphatidylinositol, and phosphatiditic acid) were reported by a commercial laboratory. A titer of 1:50 for any antibody was considered "positive". Antinuclear antibodies were also tested. A titer of 1:40 was considered "positive" by a commercial laboratory.

Patients underwent evaluation for a variety of gene mutations and were considered "positive" for inherited thrombophilia upon detection of any one of the following: heterozygous or homozygous factor V Leiden R506Q, prothrombin G20210A, or plasminogen activator inhibitor 4G/5G; homozygous methylene tetrahydrofolate reductase (MTHFR) C677T; or compound heterozygous MTHFR C677T/A1298C.

Statistical analysis of success rates was performed using Fisher's Exact test and the *t*-test.

The study solely comprised retrospective analysis of clinical data. Written informed consent for use of patient samples in research was acquired from each patient and maintained in individual patient records. Patient identifying information was maintained in accordance with HIPAA requirements.

Results

As summarized in Table 2, the mean gestational age at delivery was 39.5 ± 1.7, 37.4 ± 3.7, 38.5 ± 1.3, and 38.7 ± 1.5 weeks for groups 1, 2, 3, and 4, respectively. The mean birthweight was 3,267 ± 373, 2,654 ± 627, 3,129 ± 527, and 3202 ± 357 grams, respectively. The mean birthweight was significantly higher for group 1 *vs*. group 2 ($p = 0.006$). The mean birthweight was lower for group 2 *vs*. group 3 ($p = 0.03$) and lower for group 2 *vs*. group 4 ($p = 0.03$). There was no significant difference between C-section rate, preeclampsia rate or preterm delivery rate between any of the groups. As shown in Table 1, the different patient groups designated for purposes of the analysis were generally similar in terms of history, age, test results, and prior pregnancy history (Table 1).

As seen in Table 3, a patient's prior pregnancy history [re-

Table 3. — *Patient history and delivery outcomes.*

Group	# Cases	History category	Prior failures	Birthweight (g)	Gest. age delivery (wks)	Healthy	IUGR	Prematurity
Group 1								
NK>12%	4	RSA (≥ 2 losses)	3.8 ± 1.7 miscarriages	3363 ± 619	39.8 ± 1.0	75% (3/4)	25% (1/4)	0% (0/4)
Using IVIG	7	Mixed history	0.6 ± 0.5 miscarriages 2.0 ± 1.6 IVF failures	3200 ± 302	39.1 ± 2.1	57% (4/7)	0% (0/7)	43% (3/7)
	5	Infertility only	5.0±1.1 IVF failures	3283 ± 284	39 ± 1.6	80% (4/5)	0% (0/5)	20% (1/5)
Group 2								
NK > 12%	3	RSA (≥ 2 losses)	3.3 ± 1.5 miscarriages	2475 ± 922	36.3 ± 4.6	33% (1/3)	33% (1/3)	33% (1/3)
No IVIG	1	Mixed history	1.0 miscarriages 2.0 1.0 IVF failures 3.0 1.0 IVF failures 1.0 IVF	2296 ± 0.0	36 ± 0.0	0%(0/1)	0% (0/0)	0% (0/0)
	4	Infertility only	1.0 ± 2.0 IVF failures	2878 ± 458	38.5 ± 3.7	50% (2/4)	25% (1/4)	0%(0/4)
Group 3								
NK ≤ 12%	16	RSA (≥ 2 losses)	3.6 ± 1.5 miscarriages	3195 ± 493	38.9 ± 0.9	44% (7/16)	31% (5/16)	6% (1/16)
Using IVIG	13	Mixed history	0.6 ± 0.5 miscarriages 1.2 ± 1.8 IVF failures	3073 ± 614	38.2 ± 1.6	62% (8/13)	8% (1/13)	23% (3/13)
	3	Infertility only	5.0 ± 1.1 IVF failures	3015 ± 385	38.0 ± 1.0	67% (2/3)	33%(1/3)	0% (0/3)
Group 4								
NK ≤ 12%	4	RSA (≥ 2 losses)	3.8 ± 5.7 miscarriages	3257 ± 335	38.8 ± 1.0	75% (3/4)	0% (0/4)	0% (0/4)
Not using IVIG	5	Mixed history	0.4 ± 0.5 miscarriages (no IVF failures)	3232 ± 407	39 ± 1.9	60% (3/5)	20% (1/5)	20% (1/5)
	1	Infertility only	2.0 ± 0.0 IVF failures	2835 ± 0.0	37 ± 0.0	0% (0/1)	0% (0/1)	100% (1/1)

current (≥ two) miscarriage, infertility or mixed history] did not appear to correlate with birthweight results, except that the "recurrent miscarriage" (≥ two) subgroup of group 2 (three patients) demonstrated a somewhat lower mean birthweight (2,475 ± 922 grams) than the four patients in the "infertility" subgroup (2,878 ± 458 grams); however these subgroup numbers were far too small to develop any statistical conclusion.

Discussion

The authors investigated whether the proportion of peripheral blood NK could predict risk of low birthweight when determined prior to conception. The present data suggested that elevated NK proportion identified patients at higher risk of low birthweight but does not significantly predict other adverse parameters such as preterm delivery, C-section or preeclampsia rates. Women with an elevated proportion of NK cells who were treated with IVIg (group 1), had significantly higher birthweights than women with a similar NK proportion who did not have IVIg therapy (group 2) (p = 0.006). By contrast, birthweights were not significantly different between IVIg-treated and non-treated among women with non-elevated preconception NK cells (groups 3 and 4). These findings suggest a possible role for immune modulation in patients with preconception NK cell elevation with a prior history of recurrent miscarriage and/or infertility.

Efficacy of IVIg in pregnancy has been explained by a diversity of mechanisms. IVIg may provide soluble CD200 and soluble CD200 has been shown to be tolerogenic [54-56]. IVIg may activate Fc gamma receptors, priming dendritic-cell regulatory activity [57], and may provide anti-idiotype antibodies against autoantibodies, leading to therapeutic suppression [58-60]. IVIg preparations with a high fraction of sialylated Fc glycans may demonstrate improved immunomodulatory effect [61]. In addition to these mechanisms, IVIg has been shown to alter the proportion of peripheral blood NK cells and Th1/Th2 levels in women contemplating pregnancy [24, 62, 63]. IVIg has also been shown to improve delivery rates in patients with immunological infertility and/or recurrent miscarriage [64-67].

All patients with the proportion of peripheral blood NK cells > 12% were offered IVIg (see Materials and Methods section for IVIg protocol). Once IVIg was offered, the decision whether or not to use IVIg was made by the individual patient based on personal considerations such as IVIg cost and safety concerns. Other than differences in personal patient choice to use IVIg, group 1 (NK >12% using IVIg) and group 2 (NK >12% not using IVIg) were otherwise equivalent (Table 1).

In addition, both groups using IVIg in the present study (groups 1 and 3) were also relatively equivalent save for the preconception NK proportion used to define the patient groups. Both groups 1 and 3 averaged just over two doses of IVIg per patient (see Table 1). Fifty percent (8/16) of the patients in group 1 and 41% (13/32) of the patients in group 3 used preconception IVIg. Eighty-one percent (13/16) of the patients in group 1 and 84% (27/32) of the patients in

group 3 used postconception IVIg. Thirty-one percent (5/16) of patients in group 1 and 25% (8/32) used both used both preconception and post conception IVIg together. As explained in the Materials and Methods section, additional IVIg was given if NK proportion, cytotoxicity, and/or Th1/Th2 ratio remained elevated during pregnancy. Of the 48 patients that used IVIg in this study, only nine patients (19%) used IVIg into the second trimester of pregnancy (three patients in group 1 and six patients in group 3). For these three patients in group 1 that used later IVIg, the last doses were given at 23, 26, and 30 weeks gestational age, respectively (mean delivery weight 3582 ± 161 grams). For the six patients in group 3 that used second trimester IVIg, the last doses were given at 19, 20, 21, 27, and 32 weeks gestational age, respectively (mean delivery weight 3128 ± 705 grams). The majority of patients that used IVIg (81%) stopped IVIg after the first trimester.

The present authors did not specifically analyze patient groups based on IVIg protocol variations, as numbers were too small to conduct this type of analysis. However, determination of optimal IVIg protocol would make an interesting follow-up study. IVIg intervention in group 1 and 3 were very similar, suggesting that differences in birthweight observed between these two groups are very likely to be due to differences in proportion of NK cells. Preconception proportion of NK cells therefore, appears to be a useful marker for determining risk of delivering a low birthweight baby.

In the present study, the authors measured peripheral blood samples taken prior to pregnancy (average of 6.2 ± 5.2 months preconception). Samples were drawn at all times during the patient's menstrual cycle, as they have found that the cycle day does not significantly affect the peripheral blood NK proportion (see laboratory data in Figure 1). Functional differences in the maternal immune system existing prior to conception may "cast the die" for events following conception. Measurements taken in this antenatal period may be useful surrogates for assessing the underlying state of the maternal immune system. It should be noted that the present method for quantifying peripheral blood NK cells did not distinguish between subsets of NK cells, such as the CD56 bright and dim sub-populations nor provide an estimate of the fraction of NK cells competent for recruitment into the endometrium of early pregnancy. These additional NK correlations may warrant further study. In addition, investigation into the specific peripheral blood NK cell markers most predictive of low birthweight, examination of the immunological pathways involved with low birthweight, as well as exploration of clinical features most strongly correlated with these associations would be of additional benefit.

Conclusion

The present authors found a statistically significant difference between birthweight between women receiving IVIg therapy from those who did not amongst women with NK elevation quantified prior to pregnancy. They failed to find corresponding differences in the rates of preeclampsia and prematurity (though this sample size might have been insufficient to demonstrate these differences). These findings suggest that IVIg may be a treatment option for women at risk of a low birthweight baby and preconception NK cells elevation.

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