

# Evaluation of Fetuses in a Study of Intravenous Immunoglobulin as Preventive Therapy for Congenital Heart Block

## Results of a Multicenter, Prospective, Open-Label Clinical Trial

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**Objective.** The recurrence rate of anti-SSA/Ro-associated congenital heart block (CHB) is 17%. Sustained reversal of third-degree block has never been achieved. Based on potential reduction of maternal autoantibody titers as well as fetal inflammatory re-

sponses, intravenous immunoglobulin (IVIG) was evaluated as preventive therapy for CHB.

**Methods.** A multicenter, prospective, open-label study based on Simon's 2-stage optimal design was initiated. Enrollment criteria included the presence of anti-SSA/Ro antibodies in the mother, birth of a previous child with CHB/neonatal lupus rash, current treatment with  $\leq 20$  mg/day of prednisone, and  $< 12$  weeks pregnant. IVIG (400 mg/kg) was given every 3 weeks from week 12 to week 24 of gestation. The primary outcome was the development of second-degree or third-degree CHB.

**Results.** Twenty mothers completed the IVIG protocol before the predetermined stopping rule of 3 cases of advanced CHB in the study was reached. CHB was detected at 19, 20, and 25 weeks; none of the cases occurred following the finding of an abnormal PR interval on fetal Doppler monitoring. One of these mothers had 2 previous children with CHB. One child

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without CHB developed a transient rash consistent with neonatal lupus. Sixteen children had no manifestations of neonatal lupus at birth. No significant changes in maternal titers of antibody to SSA/Ro, SSB/La, or Ro 52 kd were detected over the course of therapy or at delivery. There were no safety issues.

**Conclusion.** This study establishes the safety of IVIG and the feasibility of recruiting pregnant women who have previously had a child with CHB. However, IVIG at low doses consistent with replacement does not prevent the recurrence of CHB or reduce maternal antibody titers.

One of the strongest clinical associations with autoantibodies directed to components of the SSA/Ro-SSB/La ribonucleoprotein complex is the development of congenital heart block (CHB) in an offspring, an alarming prospect facing 2% of mothers with these reactivities (1,2). The risk is 10-fold higher in women who have had a previously affected child (3–5). CHB carries significant mortality (20–30%; primarily fetal/neonatal) and morbidity (67% require permanent placement of a pacemaker before adulthood) (5,6). Evidence is emerging that in addition to conduction disease, 10–15% of affected offspring will have a life-threatening cardiomyopathy (7,8).

One of the most disturbing observations to emerge is the rapidity of disease progression, with advanced heart block being detected within a week of the presence of normal sinus rhythm (2). Biomarkers such as prolongation of the fetal Doppler mechanical PR interval have not convincingly demonstrated utility in predicting advanced heart block (2). Consistent with the fibrotic replacement of the atrioventricular (AV) node observed in autopsy studies of fetuses dying of CHB, sustained reversal of third-degree heart block has never been achieved (2,9). Current prophylactic and treatment strategies for CHB include maternal treatment with steroids, plasmapheresis, or sympathomimetics, as well as in utero cardiac pacing (9,10). None of them have significantly altered mortality. Accordingly, strategies aimed at preventing disease before immutable scarring ensues assume high priority. Although it is disappointing that animal models have not proven a universally causal effect of the antibodies per se, this is likely because antibodies are necessary but insufficient. Our approach to prevention considered the necessity of maternal antibody as well as consequent fetal factors in the cascade to pathogenesis.

Treatment with intravenous immunoglobulin (IVIG) has been of benefit in a variety of immune-

mediated and inflammatory diseases. The rationale for its use in CHB is based on our working hypothesis of the pathogenesis of disease. Tissue injury in the fetus is presumed to depend on neonatal Fc $\gamma$  receptor (Fc $\gamma$ Rn)-mediated transplacental passage of maternal IgG autoantibodies (11). Anti-SSA/Ro and anti-SSB/La antibodies, by binding to translocated antigen on the surface of apoptotic cardiocytes generated during remodeling of the conduction system and surrounding tissue, may inhibit the normal physiologic removal of these cells (12). Uncleared opsonized apoptotic cardiocytes may subsequently be efferocytosed by infiltrating macrophages, with release of proinflammatory and profibrosing cytokines, which transdifferentiate cardiac fibroblasts to a scarring phenotype (13,14). This scenario supports the consideration of prophylactic therapy with IVIG based on 2 presumed mechanisms of efficacy. The first exploits the saturation of Fc $\gamma$ Rn by IVIG. This should decrease fetal exposure to anti-SSA/Ro and anti-SSB/La by accelerating IgG catabolism in the maternal circulation and by decreasing placental transport (15,16). The second exploits the attenuation of anti-inflammatory responses by increasing the macrophage expression of Fc $\gamma$ RIIB (17). This would represent a downstream effect in the targeted organ.

Precedent for the use of IVIG is the encouraging report of only 1 recurrent case of CHB in 8 mothers with previously affected children (18) as well as the data from a murine study demonstrating a decrease in placental transport of human anti-SSA/Ro and anti-SSB/La antibodies following IVIG (19). Accordingly, a prospective, US-based, multicenter, open-label trial to determine the efficacy and safety of IVIG in the prevention of CHB in the children of women with anti-SSA/Ro antibodies and a previous child with neonatal lupus was initiated. Treatment consisted of 400 mg/kg of IVIG given every 3 weeks from week 12 to week 24 of gestation. The primary outcome was the development of second-degree or third-degree CHB.

## PATIENTS AND METHODS

**Study subjects.** Patients were entered into the Preventive IVIG Therapy for Congenital Heart Block (PITCH) study, a multicenter, prospective, open-label clinical trial, between January 2007 and January 2009. A total of 17 women from centers across the US signed consent forms that had been approved by the Institutional Review Board at the site of their infusion. Four mothers provided consent as participants in the Research Registry for Neonatal Lupus (New York University School of Medicine, New York, NY) to release medical records and send blood specimens, but they received study

drug as prescribed by their treating physicians, who elected to follow and adhere to the study protocol. Of the 21 women enrolled, there was 1 screening failure: this patient had a spontaneous miscarriage at 9 weeks prior to initiation of the study protocol.

All of the following inclusion criteria were required for study enrollment: 1) documentation of anti-SSA/Ro and/or anti-SSB/La antibodies; 2) a previous child with 1 of the following: (a) CHB (any degree) documented by electrocardiogram (EKG) if a live birth and/or by echocardiogram and/or histologic findings if fetal death; (b) characteristic neonatal lupus rash confirmed by photographs revealing annular lesions, dermatologic assessment, and/or biopsy findings; or (c) CHB and neonatal lupus rash; and 3) current intrauterine pregnancy of  $\leq 12$  weeks' gestation, with normal heart beat and heart structure. A patient was excluded from the study for any of the following reasons: 1) current prednisone dosage  $> 20$  mg/day or current use of dexamethasone at any dosage, 2) IgA levels below normal values for the laboratory conducting the test, or 3) presence of any structural abnormalities of the fetal heart that could cause CHB, such as L-transposition of the great arteries, AV septal defect, or heterotaxias.

Mothers could be clinically asymptomatic or could have symptoms of a rheumatic disease. Rheumatic diseases were classified according to the case report forms completed by the participating rheumatologists, obstetricians, and cardiologists performing the echocardiograms and verified by telephone interviews and review of medical records when available (by JPB, CL, and PMI). The following categories were assigned: 1) asymptomatic—if the patient denied having any clinical symptoms that would be consistent with a diagnosis of systemic lupus erythematosus (SLE) or Sjögren's syndrome (SS); 2) undifferentiated autoimmune syndrome—if there were insufficient criteria for a diagnosis of SLE or SS; 3) SLE—if 4 of the 11 criteria of the American College of Rheumatology were satisfied (20); 4) possible, probable, or definite SS—if the patient had at least dry eyes and dry mouth or only 1 of these 2 symptoms plus evidence of objective criteria in addition to autoantibodies, according to the American-European Consensus Group criteria (21); or 5) SLE and SS—if the criteria for both were met.

**Study design.** The trial was designed as an open-label study using Simon's 2-stage optimal design (22) to allow for early stopping due to absence of treatment efficacy. The first stage required 19 study subjects. If 3 or more mothers had children with second-degree or third-degree CHB, then the study would be terminated after the first stage. If this did not occur, an additional 35 mothers would be enrolled in the second stage for a total of 54 subjects. At the end of trial, the treatment would be considered efficacious if fewer than 6 of 54 mothers had a child with advanced CHB. With this design, the study had 90% power to conclude that IVIG is efficacious if the true recurrence rate with the treatment is 5%. In addition, the probability of rejecting the treatment for further study is 95% if the true recurrence rate is 19% (3,5). It should be noted that the enrollment goal for the first stage was exceeded by 2 patients because of an initial concern that there might be a screening failure due to miscarriage (which occurred in 1 case).

**Treatments and followup.** IVIG infusions of 400 mg/kg were given over 3–4 hours at 12 weeks, 15 weeks, 18 weeks, 21 weeks, and 24 weeks of gestation. Blood samples were ob-

tained before each infusion and at 28 weeks, 34 weeks, and delivery (including cord blood). Fetal echocardiograms were to be performed weekly between week 16 and week 26 of gestation and then every 2 weeks thereafter until week 34, in accordance with the protocol of the PR Interval and Dexamethasone Evaluation (PRIDE) study (2). The echocardiograms were recorded on VHS videotape or DVD/optical disk by the patient's pediatric cardiologist or obstetrician. The image copies were sent to the core fetal echocardiographic laboratory, where they were reread by one of us (DMF).

**End points.** The primary outcome was second-degree or third-degree AV block. The secondary outcomes were as follows: 1) sustained first-degree AV block, as defined by a prolonged mechanical PR interval (PR  $> 150$  msec [i.e., above the normal mean + 3SD]) (23) that did not progress to more advanced forms of AV block throughout the study and was subsequently confirmed by EKG at birth; 2) transiently prolonged mechanical PR interval; 3) any sign of myocardial injury, such as reduced contractility, tricuspid regurgitation, or effusions, without change in cardiac rate or rhythm; 4) echocardiographic densities consistent with endocardial fibroelastosis that was confirmed postnatally; 5) fetal death not related to cardiac dysfunction; 6) rash consistent with neonatal lupus; 7) prematurity, which was defined as a gestational age at birth of  $< 37$  weeks; 8) birth weight less than 10% in the context of gestational age; and 9) abnormal fluid collection in the fetus, consistent with hydrops.

**Laboratory studies.** Blood samples were separated into aliquots and maintained at  $-70^{\circ}\text{C}$ . For each patient, serial samples obtained according to the study protocol were stored until the pregnancy was completed. The evaluation of antibody titers and total IgG in all maternal blood samples and corresponding cord blood samples was completed on the same day. Titers of antibodies to anti-SSA/Ro and/or anti-SSB/La were determined by enzyme-linked immunosorbent assay (ELISA; Diamedix, Miami, FL). In this commercial test, the cutoff value for normal has been established at 19 ELISA units/ml for both SSA/Ro and SSB/La. Titers of antibodies to Ro 52 kd were determined by ELISA using recombinant Ro 52 as previously described (24). IgG levels were determined by radial immunodiffusion using a BN ProSpec automated analyzer system (Dade-Behring/Siemens Healthcare, Deerfield, IL).

**Statistical analysis.** Changes from baseline in maternal antibody titers measured at specific time points during pregnancy were evaluated with the paired *t*-test. *P* values less than 0.05 (2-sided) were considered statistically significant.

## RESULTS

**Demographic features and study population.** The demographic characteristics of the patients, including health and antibody status and obstetrical history, are summarized in Table 1. Twenty mothers completed IVIG treatments, and 19 of them gave birth. The majority of enrollees were Caucasian (80%). The maternal diagnoses at the time of enrollment in the study were as follows: 3 (15%) were classified as asymptomatic, 7 (35%) as having undifferentiated autoimmune

**Table 1.** Demographic characteristics of the 20 mothers enrolled in the study

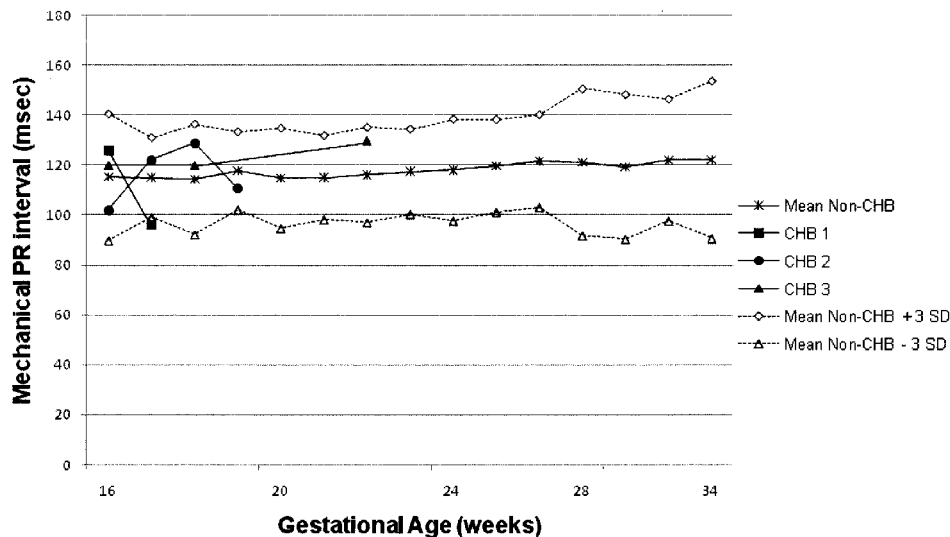
Characteristic*	No. (%) of patients
Maternal race/ethnicity	
Caucasian	16 (80)
Asian	2 (10)
African American	1 (5)
Hispanic	1 (5)
Maternal diagnosis	
Asymptomatic/UAS	10 (50)
SS	5 (25)
SS/SLE	4 (20)
RA/SS	1 (5)
Maternal obstetric history	
Previous child with cardiac neonatal lupus	18 (90)
CHB with live birth	10 (50)
CHB with fetal death	7 (35)
Fatal cardiomyopathy plus first degree	1 (5)
Previous child with neonatal lupus rash	2 (10)
Maternal antibody status	
Anti-SSA/Ro and anti-SSB/La	16 (80)
Anti-SSA/Ro only	4 (20)
Anti-Ro 52 kd	20 (100)
Maternal medications	
Prednisone $\leq$ 20 mg/day	4 (20)
Hydroxychloroquine 400 mg/day	2 (10)

\* UAS = undifferentiated autoimmune syndrome; SS = Sjögren's syndrome; SLE = systemic lupus erythematosus; RA = rheumatoid arthritis; CHB = congenital heart block.

syndrome, 5 (25%) as having SS, 4 (20%) as having SLE with secondary SS, and 1 as having rheumatoid arthritis with secondary SS. Sixteen (80%) of the patients had antibodies to SSB/La in addition to SSA/Ro. Eighteen (90%) of the enrolled patients had a previous pregnancy that was complicated by CHB (1 had 2 children with CHB), and 2 had a previous child with a neonatal lupus rash. In 8 (40%) of the mothers, the previous pregnancy complicated by CHB ended in fetal death. Four (20%) of the patients were taking prednisone at a dosage  $\leq$ 20 mg/day (mean dosage 9.5 mg/day; range 8–10 mg/day) during the study, and 2 mothers were taking hydroxychloroquine at a dosage of 400 mg/day throughout their pregnancy.

**Fetal outcomes.** Twenty mothers completed the IVIG infusions (1 woman received only 4 doses of IVIG), and 19 mothers gave birth. Seventeen fetuses completed serial echocardiograms and showed normal PR intervals (Figure 1) and had normal EKG findings at birth. Of these 17 fetuses, 1 neonate presented with a rash consistent with neonatal lupus several days after birth.

Three fetuses were diagnosed as having advanced heart block. The first case of CHB was detected at 19 weeks of gestation in a mother who had received 3 doses of IVIG at weeks 12, 15, and 18. The fetal echocardi-

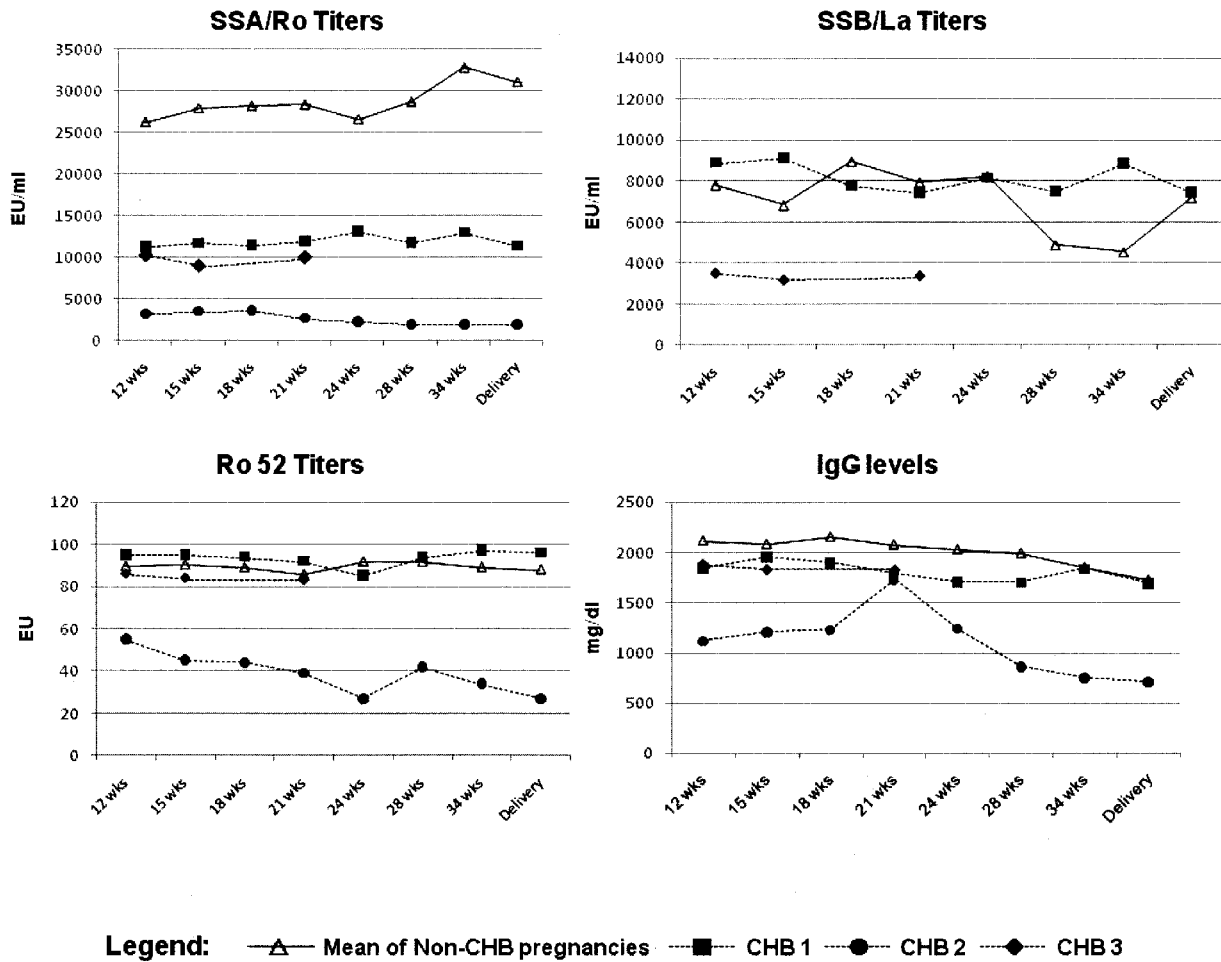


**Figure 1.** Mechanical Doppler PR interval for congenital heart block (CHB) and non-CHB pregnancies. Mean and SD values calculated for each gestational week are illustrated. For the non-CHB group, values obtained between week 16 and week 34 are shown; in addition, the mean + 3SD and the mean - 3SD in the non-CHB group are shown. PR intervals for the fetuses with CHB are all within the normal range (mean  $\pm$  3SD), and thus, first-degree atrioventricular block did not precede advanced CHB.

gram obtained at the time of detection revealed third-degree heart block with mild tricuspid regurgitation and no hydrops. The previous fetal echocardiogram at 17 weeks of gestation revealed a normal PR interval and no signs of valvular dysfunction. Once CHB was diagnosed, the patient began taking dexamethasone at a dosage of 4 mg/day for 10 days, with no effect on the fetal heart rhythm. The patient delivered at 37 weeks of gestation. The neonate was small for its gestational age, and a permanent pacemaker was placed immediately after birth. In addition, the postnatal echocardiogram showed a moderate secundum atrial septal defect, which was corrected by surgical closure at 2 months of age.

CHB was diagnosed in the second fetus at 20

weeks of gestation, after the mother had received 3 doses of IVIG. Routine weekly fetal echocardiograms were performed according to the study protocol from 16 weeks of gestation, and each showed a normal PR interval. Two weeks after the third infusion, the fetal echocardiogram revealed second-degree Wenckebach-type block, with occasional dropped beats. The mother was prescribed 1 oral dose of 4 mg of dexamethasone and received a total of 2 gm/kg of IVIG plus intravenous dexamethasone at a dosage of 4 mg/day for 2 days. However, second-degree CHB progressed to third-degree CHB within 2 days. There was no evidence of tricuspid regurgitation, effusions, or hydropic changes. The baby was born at 37 weeks of gestation and was



**Figure 2.** Antibody titers and IgG levels during pregnancy. There were no significant decreases in the titers of anti-SSA/Ro, anti-SSB/La, anti-Ro 52 kd, or IgG levels in the mothers who received intravenous immunoglobulin (IVIG) therapy. Two of the 3 women whose pregnancies resulted in a fetus with congenital heart block (CHB) received only 3 doses of IVIG. The third mother received only 4 doses. The findings in each of these 3 women are shown separately. CHB 2 did not have SSB/La antibodies. EU = enzyme-linked immunosorbent assay units.

**Table 2.** Growth and safety data in the 19 neonates

Characteristic at birth	No. (%) of children
Weight	
<10%	4 (21)
10–50%	7 (37)
51–90%	8 (42)
Length	
<10%	3 (16)
10–50%	6 (32)
51–90%	10 (52)
Head circumference	
<10%	3 (16)
10–50%	6 (32)
51–90%	8 (42)
Unknown	2 (11)

small for its gestational age. The postnatal echocardiogram confirmed third-degree CHB with normal systolic function. A pacemaker was implanted at 7 days of age.

A third CHB case was detected at 25 weeks of gestation, after the mother had received 4 IVIG infusions. Previous fetal PR intervals during the pregnancy had been normal up to 22 weeks of gestation; however, the mother missed the week 23 and week 24 fetal echocardiograms, and third-degree block was detected at 25 weeks of gestation. After discussion with her physician, the patient decided to take only 1 dose of 4 mg of dexamethasone. The fetal echocardiogram performed during her last evaluation at 30 weeks of gestation showed no signs of hydrops, tricuspid regurgitation, or effusions. The ventricular rates were above 96 beats per minute.

Two of the 3 mothers whose pregnancies were complicated by CHB were classified as having SLE with secondary SS at the time of enrollment, and the other was classified as having undifferentiated autoimmune syndrome. In addition to anti-SSA/Ro antibodies, 2 of the mothers had anti-SSB/La antibodies, and each of them also had anti-Ro 52-kd antibodies. One of these mothers had 2 previous children with CHB. The other 2 mothers had 1 previous pregnancy complicated by CHB, 1 of which ended in fetal death. The study was stopped at this point, according to the predetermined rule.

**Maternal antibody titers.** Sera obtained from mothers who completed the study were available for evaluation of anti-SSA/Ro and SSB/La titers. Antibody titers assessed before every IVIG infusion, at 28 weeks, at 34 weeks, and at the time of delivery were compared with those obtained at baseline. As shown in Figure 2, treatment with IVIG did not significantly alter the titers of anti-SSA/Ro, anti-Ro 52 kd, or anti-SSB/La antibodies ( $P > 0.05$  for all comparisons).

**Safety data.** There were no changes in maternal blood pressure and no severe headaches, rashes, fever, or any other adverse effects related to the infusions. There were no serious adverse events. Neonatal growth data are presented in Table 2. Weight, height, and head circumference were derived from gestational age-specific growth curves to correct for prematurity when necessary. Four (21%) of the newborns (2 with CHB and 2 healthy) were small for their gestational age (<10%), and 3 (16%) were born prematurely (<37 weeks of gestation). One of the mothers with a healthy baby who was small for its gestational age and 2 of the mothers with premature babies took prednisone during their pregnancy.

### DISCUSSION

This study recruited 21 mothers, 20 of whom received prophylactic IVIG to prevent autoimmune-associated CHB in their children. The study was discontinued after reaching the stopping rule of detecting 3 cases of CHB in the first 19 patients who were enrolled. There were no significant changes in the titers of antibodies to SSA/Ro or SSB/La, as determined by commercial ELISA in a central laboratory, or in the titer of antibodies to Ro 52 kd. No safety issues were raised in this pilot study.

In parallel with enrollment in the PITCH study, a European study was initiated in December 2004. The treatment protocol was identical to that used in the PITCH study. The European study was terminated after 3 cases of CHB were identified, following enrollment of 15 mothers who had previously affected children with CHB (25). Combining data generated from the 2 studies in which the prior pregnancy was CHB and not rash, there were 6 cases of recurrent CHB in 33 mothers (15 UK and 18 US), which is consistent with the recently reported recurrence rate of 17.4% (4) and confirms that IVIG at a dosage of 400 mg/kg given every 3 weeks beginning at week 12 of gestation is not effective in reducing the incidence of recurrent disease.

IVIG is approved by the Food and Drug Administration for treatment of primary immunodeficiency, idiopathic thrombocytopenic purpura, Kawasaki disease, B cell–chronic lymphocytic leukemia with hypogammaglobulinemia, pediatric infection with human immunodeficiency virus, and allogeneic bone marrow transplantation in adults (26). Several mechanisms have been posited to account for the effects of IVIG in autoantibody-mediated disorders, but none of them satisfactorily explains all of the clinical situations.

Immunomodulatory properties attributed to IVIG include down-regulation by antiidiotypic antibodies and effects on receptors for cytokines and complement (27). Administration of IVIG in a murine model was shown to inhibit autoantibody-mediated thrombocytopenia by inducing inhibitory Fc $\gamma$ RIIB receptors on macrophages (17). Recently reported data support the notion that a structure within the Fc portion of IVIG, an N-linked glycan terminating in sialic acid in 2,6 linkage to the penultimate galactose, is a requisite for activity (28). The 2,6-sialylated Fc interacts with a lectin identified as SIGN-R1, which is expressed on marginal zone macrophages, to initiate an antiinflammatory pathway that ultimately modifies the ability of effector macrophages to trigger activation via Fc $\gamma$  receptors in response to autoantibody deposition. One pathway by which this may be accomplished is through enhanced expression of Fc $\gamma$ RIIB, the inhibitory FcR, on these effector macrophages. Although the applicability of these findings to the pathogenesis of CHB is unknown, it is notable that the fully processed glycan is found in only 1–3% of IgG in IVIG.

Further experimental evidence from murine models has demonstrated that maternal administration of IVIG significantly inhibits the transplacental passage of maternal antibodies; in one case, anti-SSA/Ro and SSB/La antibodies (19) and in another, antiplatelet antibodies (16). In the latter model of fetal/neonatal alloimmune thrombocytopenia, levels of pathogenic antibodies were decreased in both the maternal and fetal circulation (16). In fact, a highly emphasized explanation unifying the beneficial action of IVIG is accelerated catabolism of IgG, which is mediated by saturation of Fc $\gamma$ Rn receptors (15,29).

Accordingly, IVIG was considered a promising candidate for the prevention of CHB based on several potential mechanisms, the first 2 of which are related to lowering or even eliminating maternal antibody in the fetal circulation (maternal perspective): increased catabolism of maternal antibody and decreased placental transport of maternal antibody. Unknown, however, is the threshold level of antibody needed to cause injury and whether IVIG can effectively lower the level below the threshold needed to bind apoptotic cells and trigger an inflammatory/fibrosing sequence. The third consideration is an effect of IVIG transported into the fetal circulation, where it might act to up-regulate surface expression of the inhibitory Fc $\gamma$ RIIB receptors on fetal macrophages, thereby decreasing secretion of proinflammatory and profibrosing cytokines (fetal perspective). Accordingly, modulation of inhibitory signaling

could be a potent therapeutic strategy for attenuating autoantibody-triggered inflammatory diseases. Highly speculative would be an antiapoptotic effect of IVIG, which would certainly be relevant to the pathogenesis of CHB, in which there is evidence that apoptosis of cardiocytes provides an essential link between antibody and fibrosis (30).

The decision to use the 400 mg/kg dose of IVIG was based on safety, efficacy, and cost issues. The replacement dose of IVIG is generally considered to be between 300 and 500 mg/kg. Consistent with the serum half-life of IgG, this dosing is repeated every 3–4 weeks to maintain a protective serum level (for review, see ref. 31). The extent to which and the timing of when IVIG might decrease placental transport of the autoantibodies are unknown, since it is acknowledged that antibody transport across the human placenta is minimal at 12 weeks of gestation. In addition, the dose we administered was based on the body weight of the mother, but clearly, the effect was not necessarily intended to achieve a lowering of the mother's total autoantibody burden. It was also hypothesized that this dose might achieve an antiinflammatory effect in the fetus, which would support the lower dose if based on the weight of the fetus and not that of the mother. The Finnish trial, which was the only published study of preventive IVIG therapy in CHB, comprised only 2 doses of 1 gm/kg (18); thus, the cumulative doses used in our study were identical.

A potential reason for the absence of observed efficacy relates to underdosing of the IVIG. Antiinflammatory doses of IVIG have been reported in the range of 1–3 gm/kg of body weight (for review, see ref. 31). The absence of a change in maternal antibody titers could be a reflection of the low dose. Precedent for IVIG lowering of antibody titers as a biomarker of effect in humans is the report of serologic (decreased antibody) and clinical remission in patients with bullous pemphigus treated with IVIG (32). Whether a significant decrease in antibody titer can be used as a reliable biomarker of drug coverage has not been established. Precedent for the use of higher doses and the presumed safety of IVIG during pregnancy are represented by studies on antiphospholipid antibody-associated fetal loss and fetal alloimmune thrombocytopenia (33,34).

A clear limitation of the study is the open-label design. The decision against randomization was made based on several assumptions. The recurrence rate was estimated at 19% based on available published data at the time the study was designed (3,5). After discussion among the coinvestigators, it was decided that a clinically meaningful outcome of therapy would be at least a

50% decrease in the predicted recurrence rate. Based on power calculations, if improvement from 19% to 10% were the case, enrollment of 261 mothers who had had a child with CHB would be required per group to complete a randomized controlled study. Even if IVIG therapy could decrease the recurrence rate to 5%, such a study would require enrollment of 97 mothers per group. Given the 2% frequency of CHB in anti-SSA/Ro-positive mothers unselected for prior disease and the average yearly enrollment of 2 patients per month in the Research Registry for Neonatal Lupus, it was deemed highly unlikely that these enrollment goals could be achieved. Indeed, the current US study took 2 years to achieve enrollment for the first phase of our study, and the European study required 4 years to enroll 15 patients. Furthermore, the likelihood that all patients would agree to randomization knowing a potentially effective therapy was being studied was deemed exceedingly rare.

In summary, this study using Simon's optimal design showed that IVIG at 400 mg/kg given on a triweekly schedule from week 12 to week 24 of gestation is ineffective as a prevention of CHB in pregnancies at risk of CHB recurrence. This finding was corroborated by the European study, which used an identical protocol. However, the results support the feasibility of recruiting high-risk patients, and given the irreversibility of advanced heart block, continued efforts at prevention are needed. These could include consideration of higher doses of IVIG to exploit an antiinflammatory effect or other approaches to the inhibition of macrophage signaling and/or fibroblast promotion of scar.

**Addendum.** Since acceptance of this article for publication, the final CHB-affected pregnancy has been completed. The boy was delivered by cesarean section at 38 weeks and weighed 3,373 gm, with a length of 53 cm. At age 5 months, the block is still third-degree, but he maintains heart rates of 100 bpm and does not require pacing. A second child in the PITCH study developed a rash at 4 months following exposure to the sun.

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#### AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved

the final version to be published. Dr. Llanos had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

**Study conception and design.** Friedman, Llanos, Izmirly, Brock, Byron, Copel, Graves, Reisner, Buyon.

**Acquisition of data.** Friedman, Llanos, Izmirly, Brock, Byron, Copel, Cumiskey, Dooley, Foley, Graves, Hendershott, Kates, Komissarova, Miller, Paré, Phoon, Prosen, Reisner, Ruderman, Samuels, Yu, Kim, Buyon.

**Analysis and interpretation of data.** Friedman, Llanos, Izmirly, Brock, Byron, Copel, Graves, Phoon, Buyon.

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