Electrocardiographic Abnormalities in a Murine Model Injected With IgG From Mothers of Children With Congenital Heart Block

Jay A. Mazel, MD; Nabil El-Sherif, MD; Jill Buyon, MD; Mohamed Boutjdir, PhD

Background—It is a widely held view that congenital heart block (CHB) is caused by the transplacental transfer of maternal autoantibodies (anti-SSA/Ro and/or anti-SSB/La) into the fetal circulation. To test this hypothesis and to reproduce human CHB, an experimental mouse model (BALB/c) was developed by passive transfer of human autoantibodies into pregnant mice.

Methods and Results—Timed pregnant mice (n=54) were injected with a single intravenous bolus of purified IgG containing human anti-SSA/Ro and anti-SSB/La antibodies from mothers of children with CHB. To parallel the “window period” of susceptibility to CHB in humans, 3 groups of mice were used: 8, 11, and 16 days of gestation. Within each group, we tested 10, 25, 50, and 100 μg of IgG. At delivery, ECGs were recorded and analyzed for conduction abnormalities. Bradycardia and PR interval were significantly increased in 8-, 11-, and 16-day gestational groups when compared with controls (P<0.05). QRS duration was not significantly different between all groups. Antibody levels measured by ELISA in both mothers and their offspring confirmed the transplacental transfer of the human antibodies to the pups.

Conclusions—The passive transfer model demonstrated bradycardia, first-degree but not complete atrioventricular block in pups. The greater percentage and degree of bradycardia and PR prolongation in the 11-day mouse group correlates with the “window period” of susceptibility observed in humans. The high incidence of bradycardia suggests possible sinoatrial node involvement. All together, these data provide relevant insights into the pathogenesis of CHB. (Circulation. 1999;99:1914-1918.)

Key Words: antibodies ■ electrophysiology ■ atrioventricular node ■ heart block
thomimetics, and in utero cardiac pacing. None have significantly altered mortality rates. 13

The timing of fetal injury is not random. Instead, it occurs during a well-defined period between 15 and 24 weeks of gestation. 14,15 The development of CHB after 24 weeks of gestation is less frequent. Advances in diagnostic or therapeu tic modalities for CHB demand an understanding of how and why the SSA/Ro and SSB/La antibodies exert their pathogenic effects during this critical period. The precise “window period” of susceptibility during gestation suggests that a developmental event may be the deciding factor for CHB to occur. To characterize these arrhythmogenic effects in vivo and to study the “window period” of susceptibility, we devised an experimental murine model for passive transfer of purified human IgG containing anti-Ro (52 kDa and 60 kDa) and anti-La (48 kDa) antibodies into the circulation of pregnant BALB/c mice.

This animal model demonstrates that ECG abnormalities in pups significantly increase when antibodies are introduced during the time the conduction system develops. This may reflect a “window period” of increased susceptibility in the murine model. Marked bradycardia was also observed, suggesting significant sinoatrial (SA) nodal involvement.

Methods

Mouse Model

Mice were used because of their demonstrated success with passive antibody transfer in the amyotrophic lateral sclerosis, myasthenia gravis, and Eaton-Lambert syndrome models. 16-18 Fifty-four timed pregnant BALB/c mice were obtained (Harlan Sprague Dawley, Indianapolis, Ind) as follows: Seven 8-day pregnant, seven 11-day pregnant, and six 16-day pregnant. Eleven 8-day pregnant, eleven 11-day pregnant, and eleven 16-day pregnant mice were obtained as controls.

The above-mentioned stages of pregnancy (term is at 19 days) were chosen for injection to target developmental milestones in the cardiac conduction system of the mouse. Embryology of the CFI-I mouse, the heart begins its development on the eighth day of gestation and is completed by day 14. 19 The conduction system matures by day 13. 20 To target pre-, mid-, and post–cardiac conduction system development milestones, 8, 11, and 16 days of gestation, respectively, were chosen for passive antibody introduction.

Purification of IgG Fractions

Immunoglobulin fractions containing IgG from 2 mothers with a CHB delivery were purified from serum by protein A-Sepharose gel separation and confirmed to be pure by electrophoresis.

SSA/Ro and SSB/La Antibodies and Immunizations

Purified antibodies were diluted in 0.25 mL of normal saline and injected intravenously into the distal tail vein of the pregnant mice with a 30-gauge needle. Broad ranges of antibody dosages were administered to maximize the pathogenic effects of immunoglobulins while minimizing the maternal autoimmune response.

Four cohorts were injected as follows: First group (n=5), 8-day timed pregnancies: 1 injected with 100 µg, 2 injected with 50 µg, 2 injected with 25 µg, and 2 injected with 10 µg; second group (n=7), 11-day timed pregnancies: 1 injected with 100 µg, 2 injected with 50 µg, 2 injected with 25 µg, and 2 injected with 10 µg; third group (n=6), 16-day timed pregnancies: 1 injected with 100 µg, 2 injected with 50 µg, 2 injected with 25 µg, and 2 injected with 10 µg; fourth group (n=33), 8-day, 11-day, and 16-day timed pregnancies (as controls). In each subset, 2 mice were injected with 10, 50, and 100 µg of control IgG from a healthy mother who tested negative to anti-SSA/Ro and anti-SSB/La components, 3 were injected with vehicle only, and 2 were not injected.

ECG Recordings

The ECGs were performed at term on the day of a normal delivery from mothers and their pups. The pups were fixed in the supine position with gentle abdominal restraint with paper tape. No anesthesia was necessary. Leads I, II, and III were recorded with limb leads attached to the pups with miniature adhesive electrodes customized for this purpose. Two adjustable heating lamps were used to maintain body temperature within a range of 36° to 37°C. ECGs from the mothers were similarly obtained with the use of a minimal inhalation anesthetic, metofane. Paper speed settings were adjusted to 25, 50, and 100 ms, with filter settings at 40 and 100 Hz. Voltage amplification was 20 mV. Tracings were analyzed for heart rate, QRS duration, and conduction abnormalities including first-, second-, and third-degree heart block. Bradycardia was defined as 40% less than controls. 21 PR prolongation was defined as >50 ms, corresponding to the mean ±2 SD (mean control PR = 40.4 and SD = 4.9 ms). 21 After ECG recordings, body weight of individual pups was assessed and compared between different groups.

Blood Collection and ELISA

Blood specimens were obtained from both mothers and pups. Blood specimens were centrifuged at 20,000 rpm for 15 minutes and analyzed by ELISA for antibody levels. ELISA was performed as previously described. 21 Recombinant SSA/Ro and SSB/La proteins were used as ELISA substrates. Typically, 60-kDa and 52-kDa SSA/Ro recombinant protein fractions were diluted in PBS, 1000-fold and 16,000-fold, respectively, for coating 96-well microtiter plates (~1 µg for the 60-kDa protein and 0.1 µg for the 52-kDa protein). Plates were incubated overnight at 4°C. The plates were then washed with PBS containing 0.05% Tween 20 (PBS-Tween) and blocked with 1% BSA in PBS-Tween, followed by incubation with human antiserum at 1:100 dilution for 1 hour. Each serum was run in duplicate. After washing, F(ab')2 goat anti-human or mouse IgG alkaline phosphate conjugate was added for 1 hour. The plates were washed again and developed with p-nitrophenyl phosphate and disodium salt in diethanolamine buffer. Results were expressed as the optical absorbance at 405 nm less reagent blank.

Statistical Analysis

Data were presented as mean±SEM and analyzed statistically with the paired Student’s t test and ANOVA. A value of P<0.05 was considered significant.

Results

Significant bradycardia and PR prolongation were present in pups of the 8-, 11-, and 16-day gestation groups when compared with controls. In contrast, pups of mothers not injected, or injected with either control IgG or vehicle, had no conduction abnormalities. Figure 1 illustrates the averaged data for heart rates and PR interval in control and injected groups.

Bradycardia was present in 70% (7 out of 10) of pups from mothers injected at 11-day gestation. The average heart rate in the 11-day group was 233.9±19 bpm (n=10), whereas control heart rates averaged 526.5±8.1 bpm (n=52, P<0.001). In pups of mothers injected at 8-day and 16-day gestation, bradycardia was present in 44% (7 of 16 pups) and 33% (10 of 30 pups), respectively. Average heart rate was...
by guest on May 2, 2017

Passive Immunization of Pregnant Mice

Figure 1. Histograms of bradycardia and PR prolongation in pups of the 8-, 11-, and 16-day gestation groups compared with control. A, Bradycardia was present in 8-, 11-, and 16-day gestation groups but most pronounced in the 11-day group. B, PR prolongation was present in 8-, 11-, and 16-day gestation groups but most pronounced in the 11-day group. In contrast, no ECG abnormalities were present in pups from control groups.

Heart Rate

PR Interval

Figure 2. Selected ECG tracings (lead I at 100 mm/s) of pups from control and from mice immunized with IgG. Example of a normal ECG is shown in A, with normal sinus rhythm (NSR) at 545 bpm and PR interval=70 ms. Tracing of pup from an immunized mouse with human IgG (from a mother whose child has congenital heart block) demonstrates marked bradycardia at 187 bpm and first-degree AV block (AVB); PR interval=70 ms in B.

Discussion

These results demonstrate that injection of pregnant mice with human IgG from mothers of children with CHB results in bradycardia and first-degree AV block but not complete heart block. In addition, ECG abnormalities are dependent on the period of gestation during which the injection was performed. This suggests that timing is critical in the development of conduction abnormalities during pregnancy.

Window of Susceptibility

In the clinical setting, conduction abnormalities occur between 15 and 24 weeks of gestation.14,15 In this model,
bradycardia and PR prolongation were observed in all 3 groups (8, 11, and 16 days of gestation) but most pronounced in the 11-day group. This suggests a critical time or “window period” during which the heart is most vulnerable to pathogenic antibodies.

Onset of the “window period” is most likely related to the earliest transplacental passage of IgG from the maternal circulation. SSA/Ro and SSB/La antibodies, like other maternal immunoglobulins, interact with the Fc receptors on the trophoblastic cell surface, are engulfed in endocytic vacuoles, are actively transported across the placenta, and are released by exocytosis into the fetal umbilical circulation. In humans, these IgGs are detectable as early as 6 to 11 weeks of gestation but increase significantly at approximately 16 to 17 weeks of gestation.

Interestingly, by 16 weeks of gestation, the human conduction system is functionally mature, coinciding with the earliest reports of CHB manifestation in the fetus. It is tempting to speculate that the fetal heart may be exposed to autoantibodies at an earlier stage of development and that its effects are manifest after the conduction system is mature. Alternatively, the conduction abnormalities may only manifest once critical levels of antibodies are present. Although the highest percentage of bradycardia and first-degree AV block in the current model occurred with the lowest dose of antibodies, this probably resulted from an increased maternal immune response to higher doses and may not reflect the clinical setting. It is well known that when large amounts of antigen/antibody such as serum are introduced into the circulation, a serum sickness–type reaction may result. The immunologic pathogenesis depends on antigen antibody concentrations. Higher concentrations of antibodies can form large complexes, which are rapidly cleared, whereas small complexes persist because they fail to activate complement receptors.

The mouse model was used to investigate the above hypotheses because the embryology of the mouse has been extensively studied. In the mouse, paired cardiac mesenchymal primordia begin fusing as early as 7 days. By 8 days, a tubular heart with the beginnings of myocardial contractions appears. The atrial and bulboventricular areas become clearly defined when the bulboventricular areas contract. By 9½ days of gestation, the interventricular septum is formed, and by 10 days of gestation, paired heart chambers may be seen. By day 12, the foramen ovale forms and the atrial and ventricular septum are almost complete. The AV node is established by day 13 of gestation. By 14½ days of gestation, the fetal circulatory system is complete and functioning. Our data revealed that first-degree AV block was most prominent in the 8- and 11-day groups, coinciding with the development of the conduction system in mice.

We previously reported the development of conduction abnormalities, including complete AV block, in pups from mothers actively immunized with a recombinant 52-kDa SSA/Ro protein. The most striking difference between the active and passive models is the absence of complete AV block in pups of the passive model. The exact mechanism is not yet clear. One explanation may be that murine autologous autoantibodies are more specific and therefore more pathogenic than passively introduced human antibodies.

Bradydcardia and SA Node Involvement

Because abnormalities of the AV node characterize human autoantibody–associated CHB, the AV node rather than the SA node was the main focus of previous publications. Although Garcia et al., by using rabbit heart, and Boutjdir et al., by using rat and human fetal heart, have also observed sinus bradycardia in their models, this bradycardia was not emphasized. The high incidence of bradycardia in the current model suggests possible SA nodal involvement. Indeed, human fetal autopsies showed calcification of the SA node, further suggesting that the SA node may also be affected. Whether the observed sinus bradycardia is related to the prolonged PR interval remains to be elucidated. Sinus brady-

| TABLE 1. Summary of ECG Parameters in Control and Immunized Pups |
|-----------------------------|----------------|----------------|----------------|----------------|
|                            | Pups, n  | Heart Rate, bpm | PR, ms | QRS, ms | Bradycardia, % | I Degree, % | II and III Degree |
| Control                     | 52      | 526.5±8.1       | 40.4±1.1 | 22.2±1.21 | 0               | 0          | 0                |
| Passive                     |         |                |        |         |                |            |                  |
| 8-day                       | 16      | 289.3±14.6*    | 62.9±2.3* | 24±0.92 | 44              | 88         | 0                |
| 11-day                      | 10      | 233.9±19.0*    | 74±4.5* | 24.5±1.25 | 70              | 90         | 0                |
| 16-day                      | 30      | 303.9±12.0*    | 57.8±2.3* | 22.8±0.52 | 35              | 45         | 0                |

*P<0.05 compared with control.

![Figure 3. Histograms of effects of different IgG concentrations on bradycardia and PR prolongation. IgG concentrations of 10, 25, 50, and 100 µg are represented. Bradycardia and first-degree heart block was present in 100% of pups from the 10-µg group, whereas higher doses caused less conduction abnormalities. This was significant (P=0.041) after accounting for the gestational day of injection.](image)

![Table 2. Blood Levels From Pups of Mice Immunized With SSA/Ro and SSB/La Antibodies as Measured by ELISA](image)
cardia that is due to intrinsic negative chronotropic effect or sinus node automaticity is not accompanied by slowing of AV nodal conduction. It could be argued that a slower input to the AV node might result in an improved cardiac velocity. On the other hand, an autonomic (vagotonic) bradycardic effect on the sinus node automaticity could also be associated with vagotonic slowing of AV nodal conduction. A vagotonic effect on the sinus node activity and AV nodal conduction secondary to the effect of autoantibodies cannot be ruled out. The exact mechanism by which autoantibodies affect the SA node would require further investigations of ion channels in the SA node.

Possible Target Epitopes

Investigators have long questioned how an intracellular Ro and La antigen can become accessible to extracellular circulating antibodies. Some hypotheses propose that the Ro or La proteins are trafficked to the cell surface during development by the induction of stress proteins, hormonal influences, viral infection, or by apoptosis.27–30 The possibility that the antigenic activity of SSA/Ro antibodies may be independent of the Ro and La ribonuclear protein has been proposed. This hypothesis is supported by the observation that the precipitin activity remains after either treatment with RNase or separation from the RNA.31 Our previous reports21,24 suggested that the arrhythmogenic effect of autoantibodies from mothers of children with CHB can be attributed partly to their interaction with calcium channels. The autoantibodies inhibited L-type calcium channels at the whole-cell and single-channel levels.21,24 If calcium channels are indeed the target epitope, this could account, at least in part, for the observed bradycardia and first-degree AV block because calcium channels are mainly responsible for electrogenesis both at the sinus node and the AV node.

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