A Serologic Marker for Fetal Risk of Congenital Heart Block

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Objective. To analyze the humoral immune response to Ro/SSA and La/SSB antigens in detail, in order to identify markers in mothers at high risk of having children with congenital heart block (CHB).

Methods. Serum samples were obtained from 9 Ro/La-positive mothers who gave birth to affected children, from their 8 newborns with CHB, and from 26 Ro/La-positive mothers whose children were healthy. Antibodies against Ro 52-kd, Ro 60-kd, and La were analyzed by enzyme-linked immunosorbent assay and immunoblotting, using recombinant proteins and synthetic peptides.

Results. IgG anti–Ro 52-kd antibodies were detected in all mothers who gave birth to children with CHB, as well as in their affected children, but were less frequent and at lower levels in control mothers. Fine mapping revealed a striking difference in which the response in mothers with affected children was dominated by antibodies to amino acids 200–239 of the Ro 52-kd protein (P = 0.0002), whereas the primary activity in control mothers was against amino acids 176–196 (P = 0.001). Furthermore, 8 of 9 mothers of children with CHB had antibody reactivity against amino acids 1–135 of the Ro 52-kd protein, containing 2 putative zinc fingers reconstituted under reducing conditions.

Conclusion. The results suggest that development of CHB is strongly dependent on a specific antibody profile to Ro 52-kd, which may be a useful tool to identify pregnant Ro/La-positive women at risk of delivering a baby with CHB. Close monitoring of mothers at high risk would enable early detection of a block that is still developing and allow early treatment to combat more serious complications.

Neonatal lupus erythematosus affects children of anti-Ro/SSA– and anti-La/SSB–positive women. These women are commonly diagnosed with systemic lupus erythematosus (SLE) or Sjögren’s syndrome, but they may be asymptomatic. Congenital heart block and cutaneous lupus are the most common manifestations of neonatal lupus; complications such as liver dysfunction, leukopenia, anemia, and thrombocytopenia also occur, but less frequently (1,2). Due to permanent injury of the cardiac conduction system, congenital heart block results in chronic heart failure, or even fetal death.

The autoimmune condition of the fetus is acquired passively when maternal autoantibodies to Ro and La are transferred over the placenta and potentially injure the developing infant. Although these autoantibodies are detected in the sera of mothers with affected children, the risk of congenital heart block in a single Ro/La-positive pregnancy is not more than 2–5% (3,4). However, recent reports indicate that minor or subclinical affection of heart rhythm is more common than previously thought (5,6). The block usually develops within the eighteenth to twenty-fourth week of gestation, and in mothers with previously affected infants, the probability of congenital heart block increases to 16–25% (3,7–9). The mortality of fetuses diagnosed with congenital heart block is high, at 10–30%, and around two-thirds of children born with congenital heart block require lifelong use of a pacemaker (10).

The disease mechanism for congenital heart
block is not completely understood, but in addition to its association with antibodies to Ro and La, signs have been reported of ongoing inflammation, such as deposits of antibodies, complement components, and lymphocytic infiltrates around the conduction system in fetal heart tissue, emphasizing the importance of the immune system in mediating tissue destruction (11). Evidence for the pathogenic role of the Ro and La autoantibodies has been provided in an antibody-specific mouse model in which female mice were immunized with recombinant Ro and La proteins, after which different degrees of block could be detected in pups (12).

To date, an established third-degree heart block is irreversible. However, treatment with corticosteroids initiated before the sixteenth week of gestation has been suggested to reduce the risk of developing congenital heart block (13). Other studies indicate that fluorinated steroids can prevent advancement to a third-degree atrioventricular block but not completely reverse the conduction defect (14,15).

These findings stress the importance of identifying the predisposed Ro/La-positive women at high risk for conceiving affected children, so that the fetal incomplete heart block can be detected before developing into an irreversible third-degree condition. For this purpose, we analyzed Ro/La-epitope specificity of antibodies in sera from mothers of children with congenital heart block, their affected children, and Ro/La-positive mothers with healthy children. Distinct epitope-recognition profiles were identified in mothers with affected children as compared with mothers of healthy children.

**PATIENTS AND METHODS**

**Patients.** Sera were obtained from 9 mothers who were diagnosed with either Sjogren’s syndrome or SLE or were asymptomatic, their 8 newborn children with different degrees of congenital heart block, and 26 Ro/La-positive mothers who gave birth to unaffected children. Diagnoses and clinical features are summarized in Table 1.

Table 1. Patients included in the study*

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of patients</th>
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<tbody>
<tr>
<td>Mothers with CHB child (n = 9)</td>
<td></td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>2</td>
</tr>
<tr>
<td>Secondary SS</td>
<td>1</td>
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<tr>
<td>SLE</td>
<td>2</td>
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<tr>
<td>Asymptomatic</td>
<td>4</td>
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<tr>
<td>Mothers with healthy child (n = 26)</td>
<td></td>
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<tr>
<td>Diagnosis</td>
<td></td>
</tr>
<tr>
<td>SS</td>
<td>9</td>
</tr>
<tr>
<td>Secondary SS</td>
<td>6</td>
</tr>
<tr>
<td>SLE</td>
<td>11</td>
</tr>
<tr>
<td>Children with CHB (n = 8)</td>
<td></td>
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<tr>
<td>Degree of block</td>
<td></td>
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<tr>
<td>I</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>1</td>
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<tr>
<td>III</td>
<td>4</td>
</tr>
<tr>
<td>Fatal block III</td>
<td>1</td>
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<tr>
<td>Pacemaker</td>
<td>1</td>
</tr>
<tr>
<td>Sibling pairs</td>
<td>1</td>
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</tbody>
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* CHB = congenital heart block; SS = Sjogren’s syndrome; SLE = systemic lupus erythematosus.

**Recombinant proteins and synthetic peptides.** Recombinant Ro 52-kd, La, and Ro 52-kd proteins and subclones were expressed as described previously (18) (Figures 1A–C). Briefly, proteins were expressed in Escherichia coli TB-1 cells using the pMAL vector (New England Biolabs, Beverly, MA) with maltose-binding protein (MaBP) as fusion partner. Wild-type MaBP was used as control. Full-length proteins and MaBP were purified on amylose columns according to the manufacturer’s instructions (New England Biolabs), and the protein concentration was determined with the Bio-Rad protein assay (Bio-Rad, Richmond, VA).

Five peptides of 21-amino acid (aa) residues, overlapping by 10 aa, with the first peptide starting at aa residue 136 of the Ro 52-kd protein, were synthesized as described previously (19), using a Tecan/Syro synthesizer (Multisynthet, Bochum, Germany). In addition, 2 longer peptides, covering aa residues 197–232 and 200–239, were synthesized (Figure 1C). Peptide purity was confirmed by reverse-phase high-performance liquid chromatography using a gradient of water/acetonitrile. Peptides were named according to the relative position of their first aa within the Ro 52-kd sequence (p136, p146, etc.).

**ELISA for detection of antibodies against Ro 52-kd, Ro 60-kd, and La.** ELISA was performed as previously described (19). Briefly, medium-binding 96-well plates (Nunc, Odense, Denmark) were coated with 1 μg per well of recombinant full-length Ro 52-kd, Ro 60-kd, or La protein diluted in carbonate buffer, pH 9.6. Plates were blocked with phosphate buffered saline (PBS)/0.05% Tween/5% milk powder, and sera were tested at a dilution of 1:1,000 in PBS/Tween/1% milk powder. Bound antibodies were detected by affinity-purified, alkaline phosphatase (AP)–conjugated anti-human IgG, IgA, or IgM antibodies (Dakopatts, Glostrup, Denmark). Phosphatase substrate tablets (Sigma, St. Louis, MO) dissolved in diethanolamine buffer, pH 9.6, were used as substrate and incubated for 30 minutes for detection of IgG, and 2 hours for
detection of IgA and IgM. The absorbance was measured at 405 nm. All steps were performed at room temperature except coating, which was performed at 4°C. Sera from healthy subjects lacking anti-Ro and anti-La antibodies were used as controls, and showed an optical density (OD) of 0.1 in all readouts. All sera were tested for antibodies to the MaBP fusion partner of the recombinant proteins, showing OD values 0.1 in all cases.

**Peptide ELISA.** The peptide ELISA was performed as previously described (19), with slight modifications. High-binding 96-well plates (Nunc) were coated with 100 µl of 10 µg/ml peptide per well in carbonate buffer, pH 9.6. Plates were coated overnight at room temperature and for at least 24 hours at 4°C before use. After this, all steps were performed at room temperature. Plates were washed 3 times with PBS/Tween and blocked with 200 µl PBS/Tween/5% milk powder per well for 30 minutes. After washing 3 times with PBS/Tween, 100 µl of patient sera, diluted 1:1,000 in PBS/Tween/1% milk powder, was added and incubated for 2 hours. Plates were washed 3 times with PBS/Tween and affinity-purified, AP-conjugated

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**Figure 1.** Schematic drawing of the recombinant Ro 60-kd, La, and Ro 52-kd proteins (solid black boxes denote RNA binding domains, grey shaded boxes represent zinc finger motifs, and the cross-hatched box shows the leucine zipper structure) and subclones used in immunoblotting and enzyme-linked immunosorbent assay (ELISA). Under each schematic drawing of subclones, a Western blot of the deletion proteins from a representative congenital heart block–associated serum (mother or child) is depicted. A, The Ro 60-kd protein. Thirteen subclones were used for mapping the antibody specificity in immunoblotting. Lane 1, Maltose-binding protein (MaBP); lane 2, Ro60-A; lane 3, Ro60-A2; lane 4, Ro60-A3; lane 5, Ro60-D; lane 6, Ro60-D1; lane 7, Ro60-E; lane 8, Ro60-F; lane 9, Ro60-G; lane 10, Ro60-H; lane 11, Ro60-H1Pst; lane 12, Ro60-I; lane 13, Ro60-K; lane 14, Ro60-L. B, The La protein. Two La fragments were used in immunoblotting. Lane 1, MaBP; lane 2, La 4-4; lane 3, LaXbal. C, The full-length Ro 52-kd protein and the recombinant fragments used in immunoblotting. Lane 1, MaBP; lane 2, Ro52-1; lane 3, Ro52-2; lane 4, Ro52-3; lane 5, Ro52-3PvuII; lane 6, Ro52-4; lane 7, Ro52-5; lane 8, Ro52-6; lane 9, Ro52-7. The immunodominant fragment Ro 52-kd-6 encoding amino acids 136–227 was further mapped using overlapping synthetic peptides in ELISA.
anti-human IgG antibody (Dakopatts), diluted 1:2,000 in PBS/Tween/1% milk powder, was added and incubated for 2 hours. Plates were then washed 3 times and phosphatase substrate tablets (Sigma) diluted in diethanolamine buffer, pH 9.6, was added. The absorbance was measured at 405 nm after 6 hours of incubation.

**Western blot.** Recombinant proteins were denatured by boiling for 5 minutes in 5% sodium dodecyl sulfate (SDS) sample buffer and run on 10% SDS–polyacrylamide gel electrophoresis (PAGE) gels. Proteins were transferred by electrophoresis onto nitrocellulose filters and blocked overnight with PBS/Tween/4% bovine serum albumin (BSA)/5% milk powder at 4°C. Thereafter, filters were incubated for 2 hours with patient sera, diluted 1:500 in PBS/Tween/1% milk powder. To detect bound antibodies, filters were incubated for 2 hours with affinity-purified, AP-conjugated anti-human IgG antibody (Dakopatts), diluted 1:2,000 in PBS/Tween/1% milk powder. Nitroblue tetrazolium and BCIP (Sigma) were used as substrate. Filters were washed in PBS/Tween between each step and all incubations were done at room temperature.

**Western blot under reducing conditions.** The assay was performed as previously described (20) with subclone Ro 52-kd-4 encompassing aa 1–135, which includes 2 putative zinc-finger motifs. Recombinant proteins were denatured by boiling for 5 minutes in 5% SDS sample buffer and run on 10% SDS-PAGE gels. Proteins were transferred by electrophoresis onto a nitrocellulose filter and incubated with a reducing buffer (100 mM Tris HCl, pH 6.8, 50 mM NaCl, 10 mM dithiothreitol) at 4°C overnight and then blocked with PBS/Tween/4% BSA/5% milk powder at room temperature for 30 minutes. The reduced filters were incubated with patient sera diluted 1:500 in PBS/Tween/1% milk powder for 2 hours. Detection of bound antibodies was performed as described above.

**Statistical analysis.** The data obtained by ELISA were analyzed using the Mann-Whitney U test or Fisher’s 2-tailed exact test (for peptide-ranking analysis), by Statistica software (version 5.5; Tulsa, OK). An α value of less than or equal to 0.05 was considered significant.

**RESULTS**

**IgG, IgA, and IgM antibody profile to Ro/SSA and La/SSB.** We investigated children with congenital heart block, their mothers, and mothers of healthy children. Sera from 9 mothers whose children were born with heart block, sera from 8 affected children, and sera from 26 anti-Ro/La–positive mothers who gave birth to healthy children were analyzed for Ro and La antibodies. The Ro/SSA antigen consists of at least 2 proteins, Ro 60-kd and Ro 52-kd, and the La/SSB antigen consists of a single 48-kd protein. All 3 proteins were expressed as recombinant antigens on ELISA and Western blot. Overall, the IgG profile of reactivity to Ro and La antigens in the children was the same as in the mothers, although the sera from the children exhibited lower specific levels.

IgG anti-Ro 52-kd antibodies could be detected in all mothers (9 of 9) who gave birth to children with congenital heart block, as well as in all 8 of their affected children. In mothers of unaffected children, IgG anti–Ro 52-kd occurred less frequently and at lower levels (P < 0.02) (Figure 2). IgG antibodies to Ro 60-kd and La could not be detected in all patients, although in mothers of affected children compared with those whose children were healthy, they were more common, and in ELISA the levels were significantly higher (Figure 2).

Specific IgA against the Ro and/or La proteins was detected in 6 of 9 sera (67%) from mothers with affected children. Mothers of unaffected children had a lower frequency of specific IgA antibodies compared with those with affected children; only 9 of 27 (33%) had detectable levels, although the total occurrence of auto-reactive IgA did not differ significantly between the groups. IgM antibodies to Ro and La were detected in low levels in a few mothers, and no IgM antibodies could be detected in the children with congenital heart block (data not shown).

**Peptide-specific antibodies associated with development of congenital heart block.** For a further exploration of the autoimmune response associated with the development of congenital heart block, we included analyses of different domains within the Ro 52-kd protein. The immune response to Ro 52-kd was investigated using protein produced from 8 different subclones in a Western blot assay. This revealed similar reactivity in all sera and pinpointed the major antigenic region to aa residues 136–239 of Ro 52-kd (Figure 1C). A distinct pattern emerged in the mothers of affected children, as well as in their children with congenital heart block. This pattern clearly differed from that observed in mothers with healthy infants, as exemplified in Figure 3.

When defined as the peptide for which the highest OD values were obtained in ELISA, antibodies to aa 200–239 of Ro 52-kd dominated in all mothers with affected children and in their children with congenital heart block (by peptide-ranking analysis), while this was the case in only 7 of 26 of the mothers with unaffected children (P = 0.0002). Instead, sera from mothers of healthy children revealed a primary reactivity against peptide 176, spanning aa 176–196 (P = 0.001). This was not the dominant antibody response in any of the mothers with affected children.
The intergroup differences in peptide reactivity were also analyzed as the percentage of the OD value representing reactivity to a single peptide compared with the cumulated sum of OD values representing reactivity to all peptides tested individually, e.g., \( \frac{\text{OD}[p200]}{(\text{OD}[p136] + \text{OD}[p146] + \ldots + \text{OD}[p200])} \) (Figure 4). Statistical analysis confirmed that the immune response in mothers with affected children was preferentially directed to peptide 200 \( (P = 0.03) \), whereas in mothers with healthy children, sera preferentially reacted with peptide 176 \( (P = 0.009) \). This was in contrast to a

Figure 2. IgG anti-Ro 52-kd (A), anti-Ro 60-kd (B), and anti-La (C) antibody levels in mothers and children. Higher levels of IgG anti-Ro 52-kd antibodies were identified in mothers of children with congenital heart block (CHB), as compared with control mothers \( (P < 0.02) \). Levels of anti-Ro 60-kd and anti-La antibodies were also higher in mothers of affected children \( (P < 0.02 \text{ and } P < 0.05, \text{ respectively}) \). OD = optical density.

Figure 3. Ro 52-kd peptide antibody levels in mothers of children with congenital heart block (A), their affected children (B), and Ro/La-positive mothers of unaffected children (C). Sera were tested in enzyme-linked immunosorbent assay with synthetic peptides. The profile of the children was similar to that of their corresponding mother, although the children had lower levels of antibody. The response of mothers with affected children was dominated by anti-p200 antibodies, while mothers with healthy children had a different profile, dominated by antibodies to p176. OD = optical density.
comparison between patients with absolute OD levels obtained with p200, in which a statistically significant difference was not observed between the groups, indicating that the quality, not the quantity, of the immune response is of critical importance in the development of congenital heart block.

Antibodies specific for the zinc finger motifs of Ro 52-kd. Previous studies have demonstrated an additional epitope constituted by a conformational epitope contained within the zinc finger motif region, situated in the N-terminal of the Ro 52-kd protein (21,22). This epitope is present only under reducing conditions, allowing correct folding of the protein. Immunoblotting under these conditions was performed using subclone Ro 52-kd-4 (Figure 1C), encompassing aa residue 1–135. Eight of 9 mothers (89%) with affected children showed antibody reactivity to the zinc finger-containing clone, while only 17 of 26 (65%) mothers with healthy children had antibodies directed against this region.

Epitope mapping of the Ro 60-kd and La protein. We also used immunoblotting to identify regions of the Ro 60-kd and La proteins specifically targeted by patient sera. To determine the epitope specificity of the anti–Ro 60-kd antibodies, 13 subclones were used (23) (Figure 1A). In all sera in which antibodies to the Ro 60-kd protein could be detected in the immunoblotting assay, aa residue 181–320 was immunodominant. Furthermore, we found that 2 mothers who gave birth to children with congenital heart block had no or very low levels of anti–Ro 60-kd antibodies. From this observation, we draw the conclusion that heart block can develop independent of the presence of anti–Ro 60-kd antibodies.

With regard to the La protein, the N-terminal region was clearly immunodominant in all sera in which antibodies could be detected. The same pattern of reactivity was detected in sera from mothers with affected children and healthy children, as well as in sera from children with congenital heart block (Figure 1B).

**DISCUSSION**

Congenital heart block is the most serious complication of neonatal lupus erythematosus, resulting in an irreversible heart blockage in the child. Cardiac injury in children with congenital heart block is presumed to arise from the active transplacental transport of maternal Ro and La autoantibodies and is irreversible after birth. Heart blocks discovered during development of the fetus have, in some cases, been successfully treated (14,15), stressing the importance of early detection of the abnormality. However, only 2–5% of women with Ro/La antibodies give birth to children with congenital heart block. In the present study, the aim was to define maternal antibody specificities to the Ro and La proteins that could be used as markers for predicting the individual risk of delivering a child with congenital heart block in women with these autoantibodies.

A thorough mapping of autoantibody specificities was undertaken, using recombinant Ro 52-kd, Ro 60-kd, and La antigens and subclones, as well as synthetic peptides. Antibodies to the Ro 52-kd protein were detected in sera from all mothers and their children with congenital heart block, while they occurred less frequently and at lower levels in mothers with healthy children. Fine mapping of the specificity of the anti–Ro 52-kd antibodies enabled us to identify distinct profiles for the 2 groups, with a dominant response to aa 200–239 in all mothers who gave birth to affected children, while antibodies against this peptide were significantly less frequent in mothers who gave birth to healthy children. Instead, sera from the latter mothers reacted primarily with epitopes contained within aa 176–196 of the Ro 52-kd protein, and we propose that the use of a ratio between the antibody titers to aa 200–239 and aa 176–196 would constitute a relevant marker for predicting high or low risk.

Although antibodies against peptide aa 200–239 of the Ro 52-kd protein could be detected in sera from all mothers with affected children, their presence is probably not in itself sufficient to induce disease, since
they also occurred in a few mothers of healthy children. An additional mechanism or fetal factor involved in developing the disease remains to be found. Such environmental or genetic influences may also explain why successive pregnancies may have different outcomes, and why a mother may give birth to a healthy child after having a pregnancy complicated by congenital heart block. The individual peptide-recognition profile and p200 levels may also be of importance, but the variability or stability of p200 and other peptide antibody levels over time remains to be investigated.

The antibody specificity in maternal sera associated with congenital heart block has not been linked to such distinct epitopes of the protein before, although the finding of a significant association between anti–Ro 52-kd antibodies and congenital heart block is in conformity with the findings of others (24–26). Moreover, profiles associated with less risk, as indicated in the present study, have not been previously described. Earlier attempts to define a specific antibody profile focusing on other and larger parts of the protein demonstrated a prevalent, but not unique, anti–Ro 52-kd antibody response (24,26–29). Also, one should note that even though an immune response dominated by reactivity to aa 200–239 correlated with development of heart block, little or no reactivity was observed in response to the overlapping peptide encompassing aa 197–232. This stresses the importance of exact choice of peptide and may explain why these possibly heart block–inducing antibodies have not been identified previously.

Our results indicate that congenital heart block can develop independent of the presence of anti–Ro 60-kd antibodies. Similar findings suggesting that antibodies to Ro 60-kd have a minor role in predicting the clinical outcome in Ro- and La-positive mothers have been reported in several other publications (8,26,30).

The 200–239 fragment of the Ro 52-kd protein includes a leucine zipper structure, a functional domain situated within a coiled-coil region in the central part of the protein. Leucine zippers are generally contained within 30-aa residues with periodic repeats of leucines every seventh residue. Leucine zippers have been described in protein–protein interactions and in dimer formations important for DNA binding (31–33). This functional domain is of hydrophilic character and likely to be of importance for the correct folding of the protein, as well as in interaction with other molecules.

Besides reactivity against the leucine zipper structure of Ro 52-kd, we found reactivity against the zinc finger motifs, in nearly all (8 of 9) mothers with affected children. These antibodies were only observed in 17 of 26 mothers (65%) whose children were healthy. Zinc fingers can mediate nucleic acid binding and dimerization, although their role in the Ro 52-kd protein remains to be clarified. Whether autoantibodies against essential domains constitute a crucial point in the pathogenic process, possibly connected with the function of the protein, or simply coincide with the functional domains possessing a higher antigenicity must still be a matter for speculation.

By analyzing IgG, IgA, and IgM antibody isotypes to the Ro and La antigens, we found that IgG dominated the response in nearly all sera analyzed. Earlier serologic studies focusing on the IgG isotypes have shown that all 4 IgG subclasses of anti-Ro and anti-La antibodies cross the placenta, including complement-fixing subclasses, but no significant connection was identified between subclass profiles and disease in these maternal sera (34).

Although the association between congenital heart block and autoantibodies is well established, the direct effect is unclear. The Ro 52-kd is expressed in normal fetal tissues, including the heart, during gestation weeks 16–18 (35) and signs of ongoing inflammation have been observed in hearts from diseased fetuses, with deposition of antibodies and complement components as well as fibrosis and calcification replacing the atrioventricular node (11,36,37). However, Ro 52-kd is an intracellular antigen and its exposure in the fetal heart tissue remains to be clearly delineated. Accessibility to the immune system might be explained by an increased protein expression during fetal development or by incorrect protein transport and/or sorting, or might possibly be connected with apoptotic events taking place in the fetal organogenesis and development of the conduction system. It is also possible that the antibodies cross-react with another protein, as suggested recently by Eftekhari et al, who identified cross-reactivity with a cardiac receptor (38). Ro 52-kd, Ro 60-kd, and La lack any sequence homology, and the reason why autoantibodies to these distinct, nonhomologous antigens so frequently coexist is not completely understood. Mechanisms such as cross-reactivity, epitope spreading, and molecular mimicry as well as apoptosis in fetal tissue during development have been proposed (39). However, our results indicate that Ro 52-kd is the major antigen in the development of congenital heart block, and suggest it to be an important inducer in the pathogenic process of congenital heart block.

In summary, an antibody profile dominated by a response against aa 200–239 (p200) indicates the risk of a pregnancy being complicated by congenital heart block.
block, while a response to aa 176–196 (p176) indicates a low risk. A ratio between the p200 and p176 antibody levels in the pregnant woman might constitute a relevant marker for predicting high or low risk of congenital heart block in the fetus. A suggestion when identifying a pregnant woman with high risk would be to perform frequent serial echocardiograms during susceptible weeks in order to monitor fetal progress and thereby increase the chance of detecting the possible heart block early. This will raise the possibility of preventing or reversing the block with medical treatment. However, further and prospective studies will be needed to confirm and validate the use of Ro 52-kd peptide antibody profiles in predicting the occurrence of congenital heart block.

ACKNOWLEDGMENT

We gratefully acknowledge the technical assistance of Ms Evi Mellquist.

REFERENCES