

# Neonatal Lupus Syndrome: The Heart as a Target of the Immune System\*

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## ABSTRACT

Neonatal lupus erythematosus (NLE) is an auto-immune disease related to systemic lupus erythematosus (SLE). Unlike SLE it is not a spontaneous syndrome but rather an acquired one. In NLE the most common disease manifestations are a transient cutaneous lesion and cardiac conduction disturbances. The cutaneous lesions and other non-cardiac manifestations of NLE are transient and disappear about six months after birth, at the time when maternal antibodies disappear from the neonatal circulation. This fact suggests that maternal antibodies may cross the placenta leading to an inflammatory reaction in the fetal tissues. NLE is the principal cause of atria-ventricular block, when it is not associated with congenital birth defects. All the clinical studies to date correlate the heart block in NLE with the presence of certain types of circulating maternal antibodies, against the Ro/SSA nuclear proteins, in the serum of the newborn. In this paper we discuss animal models that have been developed by our and others groups to study the participation of the anti-Ro/SSA antibodies in the pathogenesis of the cardiac conduction blockades that occur in NLE.

**Key words:** neonatal lupus, auto-immunity, heart blockade, anti-Ro/SSA antibodies.

## AUTOIMMUNITY

The primordial function of the immune system in higher organisms is to activate defensive immunological reactions against microbial infections. Cells of the immune system recognize structures and molecular signals that are distinct from the body's own and an immune response ensues. However the immune system must defend the organism without destroying self-constituents and to avoid auto-reactions this system has elaborated different mechanisms. First, it has humoral and cellular effector mechanism that specifically recognize foreign

structural components with minimal cross-reactions to self-antigens. Second, it has suppressor mechanisms that inhibit the immunological reactions to self-constituents (Sudhir P 1999). Although the immune system has the properties required to avoid auto-reactions, sometimes the multiple immunological effector mechanisms undergo dysfunction leading to the development of auto-immune diseases.

The initiation of auto-immune diseases in humans can be triggered by different factors: genetic, environmental, immunological, hormonal or infectious. The main genetic factor is the major histocompatibility complex (MHC) which is involved in the maturation of T cells and in the induction of the immune reaction to protein antigens. Environmen-

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tal and infectious agents are immediate triggers of disease. In the infectious process auto-immunity is not due to the infectious agent itself, but rather to the immune response activated or deregulated by this agent. Hormonal factors may be responsible for the difference in the frequency of autoimmune syndrome occurrence between men and women; for example, the lupus erythematosus syndrome is much more frequent in woman.

The auto-immune process is the result of the loss of balance between the maintenance of immunological defense and the suppression of destructive self-reactivity. Most human auto-immune diseases are complex, involving a number of antigens of the target tissue. This may be owed to epitope spreading or to the presence of antibodies to a number of unrelated, organ specific antigens (Rose *et al.* 1996). Based on adoptive transfer studies in experimental animals there is a strong evidence that T-cell mediated immunity is very important in the pathogenesis of many auto-immune diseases (Katz *et al.* 1995), although these studies can not totally exclude the role of antibodies in the development of these syndromes (Rose & Hill 1996).

The mechanism responsible for the pathogenesis of auto-immune syndromes is the production of a large number of auto-antibodies and auto-reactive T cells. The auto-immune syndromes can be classified as spontaneous or induced, systemic or organ-specific. When an exogenous stimulus triggers the auto-immune process the syndrome is induced, as can be observed in rheumatic fever, a pathological process observed after Streptococcus infection. An example of spontaneous syndrome is lupus erythematosus. In this syndrome and in other connective tissue related diseases there is no evidence for the role of etiologic agents in the development of the disease. The auto-immune diseases classified as organ-specific are characterized by the involvement of only one organ and the auto-antibodies or auto-reactive T cells react against organ-specific antigens. Hashimoto's thyroiditis and Diabetes mellitus are examples of organ-specific auto-immune syndromes. In the systemic auto-immune diseases

auto-antibodies against antigens spread out throughout the body can be observed, and tissue damage is disseminated. In these systemic syndromes, the auto-immune disfunctions may involve skin, kidneys, joints and muscles.

Systemic lupus erythematosus (SLE) is an example of spontaneous systemic auto-immune syndrome. SLE is the most studied systemic syndrome. It is a inflammatory syndrome which results from an immunologic disturbance that may lead to or be due to the activation of polyclonal B cells, that induce the production of a large number of auto-antibodies. Abnormalities in T cells and in the antigen-presenting cells have also been observed in patients with disease in activity. Until now it is not clear if the tissue lesions are due to T cells that infiltrate the organ or to the auto-antibodies in the serum. As mentioned before there is a prevalence of this syndrome in women (10:1 women for man). In SLE there is an involvement of multiple organs (kidneys, heart and nervous system), and cutaneous lesions and joint pain are common. Alterations in cardiac electrogenesis are very rare in adults with LES (Bilazarian *et al.* 1989) but there is an association between anti-phospholipids antibodies and heart valve disease in SLE (Khamashta *et al.* 1990). Complete heart block (CHB) is a rare complication of SLE in adults, in contrast to neonatal lupus, in which CHB is a frequent disease manifestation; no more than 10 cases of CHB in SLE have been reported up to date.

#### THE NEONATAL LUPUS SYNDROME

Neonatal lupus erythematosus (NLE) is another auto-immune disease related to SLE. Unlike SLE it is not a spontaneous syndrome but rather an acquired one. In NLE the most common disease manifestations are a transient cutaneous lesion and cardiac conduction disturbances. The cutaneous lesions and other non-cardiac manifestations of NLE are transient and disappear about six months after birth, at the time when maternal antibodies disappear from the neonatal circulation. This fact suggests that maternal antibodies may cross the placenta leading to

an inflammatory reaction in the fetal tissues. The factors determining whether babies will develop heart block, skin disease, or both, are not yet known.

NLE is the principal cause of total atria-ventricular block, when it is not associated with congenital birth defects. Some children may develop a partial or total atria-ventricular block during fetal life or in the neonatal period (Sontheimer & McCauliffe 1990). In general this manifestation is irreversible, and may lead to death. All the clinical studies to date show that the heart block in NLE is associated with the presence of certain types of circulating maternal antibodies, against nuclear proteins, in the serum of the newborn. In essentially all cases of NLE associated CHB, auto-antibodies to the Ro/SSA protein complex are present. The Ro particles are ribonucleoproteins which are composed of at least two polypeptides, one with 52kD and another with 60kD, both of them associated with small RNAs in the nucleus (Mamula *et al.* 1987, Ben Chetrit *et al.* 1988). There is no homology between the amino-acid sequences of these two proteins, and they have distinct antigenic regions. Antibodies are directed to the 60-kD Ro/SSA and the 52-kD Ro/SSA proteins, although there is a prevalent reaction against the 52-kD Ro/SSA in serological analyses of mothers of children having NLE (Buyon *et al.* 1993). The 52kD polypeptide is alternatively spliced, yielding an isoform that lacks amino-acids 169-245 of the full-length protein (Chan *et al.* 1995). Although their function is not yet known, both the 60 and the 52 kD Ro have zinc finger motifs capable of binding DNA and regulating gene expression (Chan *et al.* 1991, Itoh *et al.* 1991). The anti-La/SSB is another auto-antibody found in the serum of mothers and affected children. The La protein is composed by a polypeptide of 47 to 50 kD (Habets *et al.* 1983) and is associated to 7S, 5S and tRNAs (Lerner & Steitz 1979, Rinke & Steitz 1982, Chambers *et al.* 1988). The La protein has been described as a transcription termination factor of RNA polymerase III (Gottlieb & Steitz 1989).

The pathogenesis of CHB in NLE is attributed to an inflammatory process induced by the reactivity

of these antibodies with the fetal heart and its conduction system (Lee *et al.* 1987). Bear in mind that the antibodies reach the fetal circulation via transplacental transport and that it is the maternal antibodies that react with the fetal tissue. Interestingly, although the mother is the source of the antibodies that induce the clinical manifestations characteristic of NLE, adults with SLE rarely suffer the cardiac conduction blockades seen in NLE. In a well studied case Mevorach *et al.* (1993) described an adult patient with SLE whose major symptoms were CHB and seizures. The association between those alterations and an auto-antibody mediated mechanism came from the presence of anti-Ro/SSA and anti-La/SSB auto-antibodies in the serum and cerebrospinal fluid of this patient.

Isolated complete heart block occurs in about 1 out of 20.000 births (Michaelsson & Engle 1972). Many studies have shown that anti-Ro/SSA auto-antibodies are the largest single risk factor for the development of CHB in the neonate, in spite of the maternal disease status (Watson *et al.* 1984). CHB is generally detected between 18-24 weeks of gestation (Buyon *et al.* 1998), and may be associated with myocarditis. Although anti-Ro/SSA and/or anti-La/SSB antibodies are present in more than 98% of the mothers of NLE associated CHB patients, the reported risk of having a child with CHB for anti-Ro/SSA positive mothers is only 5% (Smeenk 1997). Meilof *et al.* (1993) have suggested that the presence of these auto-antibodies are not the only determinant factor to predict the occurrence of CHB. Indeed, the role of anti-Ro/SSA and/or anti-La/SSB in CHB is not clear. It has been suggested that these antibodies react with their corresponding antigens on the surface of the cells of the conduction system in the heart (Horsfall *et al.* 1991). Although anti-Ro/SSA antibodies have been eluted from affected fetal cardiac tissue (Reichlin *et al.* 1994), there is no reason to expect the expression of the Ro/SSA protein, a nucleoprotein, in the surface membrane of the cardiac cells. In that regard it is interesting to note that Miranda *et al.* (1998) have shown that apoptotic human fetal cardiomyocytes express Ro and La anti-

gens in the surface. Even though apoptotic cells do not evoke inflammatory reactions the authors speculate that maternal antibody binding to the antigens expressed at the surface of the apoptotic cells might trigger leukocyte migration and cytokine liberation, characterizing a pro-inflammatory response that could affect the healthy cardiomyocytes surrounding the apoptotic ones. Another possibility for the tissue lesions induced by the maternal antibodies is a cross reaction between the auto-antibodies to Ro and La and a non-related protein expressed on the surface of the cardiac cells. Below we present evidence in favor of this hypothesis.

#### EXPERIMENTAL MODELS FOR NLE

Researchers have attempted to create experimental models of neonatal lupus in order to study the role of the auto-antibodies in altering cardiac electrogenesis. Based on clinical data that indicate an association between CHB in neonates and the presence of anti-Ro/SSA antibodies in the serum of the affected babies, Alexander *et al.* (1992) tested the effect of serum positive for these antibodies in papillary muscles of rabbit hearts. They demonstrated that superfusion of newborn rabbit ventricular papillary muscles with IgG-enriched fractions from anti-Ro/SSA positive sera induces a reduction in action potential duration. They further reported that the effect was only present in the IgG enriched fraction from these sera, that hearts from adult rabbits were unaffected by the anti-Ro/SSA- positive sera, and that sera from lupus patients with other antibody profiles did not alter action potential duration in newborn rabbit hearts.

We showed for the first time that the presence of anti-Ro/SSA or anti-Ro/SSA and anti-La/SSB antibody activity in IgG fractions from lupus patients' sera can induce cardiac conduction disorders similar to those observed in neonatal lupus (Garcia *et al.* 1994). Using isolated adult rabbit hearts, we demonstrated that these IgG fractions induced disturbances in the conduction of the cardiac impulse similar to those observed in neonatal lupus in vivo.

To further characterize the cellular mechanisms involved in the conduction disturbances in the whole hearts, experiments with ventricular myocytes isolated from young rabbit hearts studied by whole cell patch-clamp technique were performed. In these experiments we observed a mean reduction of 32% in the peak slow inward current, essentially a L-type calcium current, when the cells were superfused with anti-Ro/SSA positive IgGs. The results in isolated ventricular myocytes correlated well with the observations at the whole heart level, since the conduction in the A-V node is basically dependent on calcium electrogenesis. Our results were later confirmed by Boutjdir *et al.* (1997) using whole hearts and cardiac myocytes derived from human fetuses.

We also investigated the ability of affinity purified anti-52 kD Ro/SSA antibodies from patients without obstetric history of neonatal lupus to cause heart block in our rabbit heart model (Viana *et al.* 1998). Affinity purified antibodies were obtained from two sera previously known to induced A-V block by affinity chromatography using a column containing the full-length 52 kD Ro/SSA fusion protein. Paired eluate and effluent, devoid of anti-52 kD activity, from the same patient were individually perfused in whole hearts. The ability to cause A-V block was restricted to the affinity purified anti-52 kD eluates. In addition, three affinity purified anti-52 kD eluates from three IgG fractions that primarily failed to induced cardiac conduction block remained ineffective. The two sera that induced conduction blockade in these experiments were obtained from mothers with healthy infants. In contrast to our rabbit model, Boutjdir *et al.* (1997) could only see AV conduction block or reduction in calcium currents in the human fetal hearts when using anti-52 kD affinity purified antibodies from mothers who had children with CHB. The reasons for this discrepancy are not immediately obvious, but we speculate that the target protein(s) at the cell membrane may not be 100% homologous in fetal human and adult rabbit hearts. The challenge now is to unravel the identity of the antigen recognized at the surface of the cardiomyocytes. The obvious candidate, the L-type

calcium channel, has no sequence homology with the Ro protein.

Curiously, the animal model mentioned above reproduces the variability seen *in vivo*; i.e., only a small percentage of the 52 kD positive mothers have children with CHB. This is also true for another experimental model now used in the search for the pathological mechanisms underlying the neonatal lupus syndrome; the immunization of mice with recombinant Ro and La proteins. Only a fraction of the pups born from Ro and/or La positive mice exhibited CHB. Actually the first report of CHB in mice to appear in the literature used the monoclonal anti-DNA idiotype 16/6 to immunize Balb/c females (Kalush *et al.* 1994). The antibody profile in the immunized females was very complex, including antibodies reactive not only to the Ro and La proteins but also to 16/6 Id, single and double stranded DNA, Sm, RNP and cardiolipin, making the interpretation of the results very difficult. Immunization of Balb/c females with recombinant La, 60kD Ro and the two isoforms of the 52 kD Ro, also resulted in various degrees of AV conduction blockade in the pups (Miranda *et al.* 1998). Unfortunately, even in this case, antibody production is not restricted to the injected recombinant protein, since auto-antigen spreading seems to be a rather common finding with these proteins (Deshmukh *et al.* 1999, Farris *et al.* 1999, Mason *et al.* 1999). Nonetheless, the occurrence of CHB in animal models renders this syndrome much more amenable to study, and we anticipate that major progress in unraveling the physiopathology of this disease will be achieved in forthcoming years.

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