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Role of Calcium Channels in Congenital Heart Block

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Abstract

Congenital heart block (CHB) is a conduction abnormality that affects hearts of fetuses and/or newborn to mothers with autoantibodies reactive with the intracellular soluble ribonucleoproteins 48kD La, 52kD Ro, and 60kD Ro. CHB carries substantial mortality and morbidity, with more than 60% of affected children requiring lifelong pacemakers. Several hypotheses have been proposed to explain the pathogenesis of CHB. These can be grouped under three main hypotheses: Apoptosis, Serotoninergic and Ca channel hypothesis. Here we discuss these hypotheses and provide recent scientific thinking that will most likely dominate the future of this field of research.

Introduction

CHB is a conduction abnormality which affects the sino-atrial (SA) node, the atrioventricular (AV) node and the ventricles of the fetal heart with no structural abnormalities. Maternal autoantibodies' (anti-Ro/La antibodies also referred to as positive IgG) effects on the SA node are manifested as sinus bradycardia (sometimes transient), on the AV node as different degrees of AV block and on the ventricles as heart failure.

Various degrees of AV block as well as bradycardia represent the clinical findings in CHB children. Third degree AV block is irreversible with mortality approaching 30%. Some of the reported CHB deaths are due to heart failure [1,2]. Recently, a previously under-appreciated sinus bradycardia unrelated to AV block was reported in CHB animal models [3,4]. This was subsequently confirmed by Brucato et al., [5] and Hamilton et al. [6], who reported sinus bradycardia in infants born to mothers seropositive to anti-Ro positive maternal antibodies. The high incidence of sinus bradycardia in mouse CHB models and in affected infants indicates that the spectrum of conduction abnormalities in CHB extends beyond the AV node to also affect the SA node. Further support for this hypothesis comes from autopsies showing calcification of the SA node in CHB human fetal heart [7]. Because AV block has been the hallmark of CHB, the AV node, rather than the SA node, was then the main focus of previous publications [3,4,8] and perhaps even during clinical diagnosis of CHB [5]. The electrophysiological basis of sinus bradycardia has been recently established by the demonstration that maternal antibodies inhibition of L-type Ca current, $I_{Ca\text{-}L}$ [9,10], and the T-type Ca current, $I_{Ca\text{-}T}$ in the sinus node myocyte [9]. Interestingly, the potassium current, I_K, and pacemaker current, I_f which are both involved in the sinus node automaticity were not affected [9]. Maternal antibodies' inhibition of both $I_{Ca\text{-}L}$ and $I_{Ca\text{-}T}$ in the sinus

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node leads to a slower slope of phase 4 depolarization thereby resulting in a slower heart rate [9].

AV block is the most serious and irreversible manifestation of CHB. The non cardiac manifestations of CHB are skin rash, cytopenias and hepatitis [11]. All manifestations except CHB are transient and resolve at about 6 months with the disappearance of maternal autoantibodies from the neonatal circulation [12,13]. The transient features reflect the effect of the autoantibodies in organs that have the capacity of continual regeneration. Interestingly, despite being exposed to the same autoantibodies, no complete AV block has been reported in the mother's heart [14–16].

Histology

The pathogenic autoantibodies causing CHB are associated with progressive destruction of the AV node [17,18] and calcification of the SA node in human fetal heart [7]. Autopsy revealed fibrosis and calcification of the AV node [7,19]. Histological studies demonstrated antibodies in cardiac tissue [7,20]. Deposition of complement, lymphocytic infiltrates, calcification and fibrosis has also been found in fetuses dying from CHB [7,19–21]. CHB fetal hearts eluates were found to contain autoantibodies to SSA/Ro 52 and 60 kDa [22]. The observation of antibody deposition, fibrosis and calcification has been found in the entire myocardium and not restricted to the AV node [7,20,23,24]. The overall effects these autoantibodies include myocarditis and dialated cardiomyopathy [17,25–28]; a feature found in few cases of CHB. Prolongation of the QT interval has also been described [29,30].

Incidence

Because of the rarity and complex etiology of CHB, the incidence is not well established. A generally accepted mean incidence is 1:17,000 in the 1970s [31] and 1:11,000 in the latter decade [32,33]. However, this incidence dramatically increases to about 5% in Lupus patients and to 18% in subsequent pregnancies [34]. This indicates that the incidence of CHB in the latter decades [32,35] was higher than previously reported likely due to more effective detection of CHB during pregnancy using fetal ultrasound and to the improved diagnostics. The recurrence rate is 18%, [32,36,37] supporting the need for close echocardiographic monitoring in all subsequent pregnancies with heightened surveillance between 18 and 24 weeks of gestation. Despite the persisting antibodies, such a recurrence level indicates that the antibodies are necessary but other fetal factors are involved in the susceptibility to CHB.

Mortality and Morbidity

The mortality rate of 20% in CHB children of mothers seropositive for anti-SSA/Ro and anti-SSB/La has been reported [34]. The survival is proportional to the gestational age at birth; as such, the children born at a later gestational age had a lower mortality. Infants who survive the neonatal period have an excellent prognosis. In fact, more than 60% of affected children require lifelong pacemakers before entering adulthood [32,38]. Most deaths reported thereafter seem to be related to heart failure [39]. Therefore, most deaths in CHB occur in utero or in the first 3 months of life leading to a mortality of 20%.

Proposed hypothesis of CHB

Based on the facts that there is no convincing evidence that maternal antibodies can cross the sarcolemma of a normal cardiac myocyte, two categories of mechanisms for CHB have been put forward. The first is abnormal surface expression of intracellular Ro/La antigens and the second is the cross-reactivity of maternal antibodies with targets other than Ro/La antigens. To explain the pathogenesis of CHB based on these two categories of mechanisms,

three major hypotheses have been proposed. The apoptosis hypothesis [cell surface expression of the Ro/La antigen] [40], the serotoninergic hypothesis [41] and the Ca channel hypothesis [cross-reactivity of maternal antibodies with cell surface receptors and Ca channel proteins respectively] [42].

Apoptosis Hypothesis

The apoptosis hypothesis proposes abnormal (opsonization) surface expression of intracellular Ro/La antigens which become accessible to circulating maternal antibodies on the surface of the cardiac myocytes. It is suggested that opsonization converts the physiologic process of apoptosis to circumstances in which an inflammatory component is evoked [43]. Miranda et al., [40,43] demonstrated that induction of apoptosis results in surface translocation of Ro/La antigens in human fetal cardiac myocytes.

To examine whether antibodies reactive to Ro/La antigen system indeed bind to the surface of human fetal myocytes and to assess the consequences of this surface bindings, the authors used biotinylation of cell surface proteins, scanning electron microscopy of immunogold labeled cells and examined the consequences of the release of the inflammatory cytokine [43]. They confirmed that induction of apoptosis results in surface accessibility of all Ro-La antigens for recognition by tumor necrosis factor (TNF) from macrophages co-cultured with apoptotic fetal cardiac myocytes. It was concluded that opsonized apoptotic fetal cardiac myocytes promote an inflammatory response by macrophages causing damage to surrounding conducting tissue. Clancy et al. demonstrated that healthy fetal cardiocytes are involved in physiologic clearance of apoptotic cells and that surface binding by anti-SSA/Ro and -SSB/La antibodies inhibits uptake of apoptotic cells by the healthy cardiocytes. The result is accumulation of apoptotic cells promoting further inflammation and subsequent scarring [44].

It is however difficult to correlate the above findings to AV conduction abnormalities seen in CHB because the experiments were performed in ventricular myocytes, not in conduction system myocytes such as AV node myocytes. However, evidence for the subcellular translocation of La autoantigen during physiologic apoptosis in the fetal mouse conducting system has been provided [45]. Other experimental evidence has been proposed to account for the translocation of Ro/La to the cell surface, including viral infection [46], UV light and interferon (IFN) treatment [47].

Serotoninergic Hypothesis

Accessibility of intracellular target antigens to circulating maternal antibodies remains a challenge. This lead to the consideration of alternative hypotheses such as cross-reaction between one or any of the Ro-La components and a cell surface cardiac receptor and/or channel. Indeed, support for this second hypothesis is the report by Eftekhari *et al.* that antibodies reactive with the serotoninergic 5-hydroxytryptamine (5-HT4A receptor, cloned from human adult atrium, also bind 52kD Ro [41]. Moreover, affinity-purified 5-HT4 antibodies antagonized the serotonin induced L-type Ca channel activation in human atrial cells [41]. The finding that functional beta-adrenoceptors in experimental animals appear late in the gestation stage, makes this hypothesis more intriguing, since 5-HT4 receptors could functionally replace the beta-adrenergic receptor during development. Two peptides in the C terminus of 52kD Ro, aa365-382 and aa380-396, were identified that shared some similarity with the 5-HT4 receptor. The former was recognized by sera from mothers of children with neonatal lupus and it was this 52kD Ro peptide that was reported to be cross reactive with antibodies to peptide aa165-185, derived from the second extracellular loop of the 5-HT4 receptor [41]. These findings are of particular importance, since over 75% of serum from mothers whose children have CHB contain antibodies to 52kD Ro. Given the

intriguing possibility that antibodies to the 5-HT4 receptor might represent the hitherto elusive reactivity which could directly contribute to AV block, we initiated a study to determine the prevalence of anti-5-HT4 antibodies in mothers whose children have CHB [48]. RT-PCR was employed to examine the mRNA expression of the 5-HT4 receptor in the human fetal heart. One hundred sixteen sera were evaluated for anti-5 HT4 reactivity. The biochemical results demonstrated mRNA expression of the 5-HT4 receptor in the human fetal atrium. The electrophysiologic studies established that human fetal atrial cells express functional 5-HT4 receptors [48]. Moreover, sera from 116 mothers, whose children have CHB, were evaluated. Ninety-nine (85%) of these maternal sera contained antibodies to Ro, 84% of which were reactive with the 52kD Ro component by immunoblot. However, none of the 116 sera were reactive with the peptide spanning aa165-185 of the serotoninergic receptor. Due to discrepancies between studies involving reactivity of maternal antibodies and the 5-HT4 receptor, a reassessment was carried to assess the prevalence of anti-5-HT4 receptor autoantibodies in mothers of affected children [49]. This study showed that only 16% of the sera from mothers of children with congenital heart block were positive for anti-5-HT4 receptor autoantibodies. The discrepancy was attributed to differences in pH and epitope exposure in the ELISA plates. Nonetheless, it was concluded that additional risk factors are needed to contribute to the development of CHB. Although 5-HT4 receptors are present and functional in the human fetal heart, maternal antibodies to the 5-HT4 receptor were only rarely present in sera from affected children and do not seem to be strongly associated with the development of CHB [48].

Ca channel Hypothesis

The formulation of Ca channel hypothesis was driven by the fact that AV node electrogenesis is under the control of L-type Ca Channel which is responsible for conduction between the atria and the ventricle. Inhibition or blockade of this channel ultimately leads to AV block reminiscent of conduction abnormalities seen in CHB. The Ca channel hypothesis states that circulating maternal antibodies recognize L-type Ca channel pore forming protein α1-subunit to which they bind and prevent Ca ions from entering the myocyte [42]. Consequently, conduction of the SA impulse through the AV node to the ventricle will be hampered leading to delay in conduction or worst to complete AV block.

Voltage Gated Calcium Channels

Two L-type Ca channels are expressed in the heart are: Cav1.2 (α1C) and Cav1.3 (α1D). Cav1.2 or α1C is ubiquitously expressed in the heart and essentially mediates cardiac excitation-contraction coupling. Cav1.3 or α1D on the other hand, is restricted to the supraventricular tissue in the adult with the highest expression in the SA node and AV node. Cav1.2 $I_{Ca\text{-}L}$ activates at more positive (−40 and −30 mV) potentials, and accounts for the conduction electrogenesis at the AV node, whereas Cav1.3 $I_{Ca\text{-}L}$ activation occurs between −60 and −40 mV at a range in which diastolic depolarization at the SAN operates [50]. Thus, Cav1.3 $I_{Ca\text{-}L}$ is essential for normal cardiac pacemaker activity. In fact, genetic deletion of Cav1.3 exhibits sinus bradycardia and AV blocks similar to what is reported in CHB [51]. It is therefore logical that Ca channel blockade at the SA and AV nodes by maternal anti-SSA/Ro –SSB/La antibodies will be expected to interrupt the sinus rhythm and conduction of the impulse to the ventricles.

Experimental models of congenital heart block

There are a number of in vivo and ex vivo models developed to understand the mechanism and the pathogenesis in CHB. The in vivo models are divided into passive and active immunization of female mice, rats or rabbits. The ex vivo studies reproduce CHB by adding maternal autoantibodies to Langendorff perfused hearts. This section will describe each

model used with an overview of the data and their success in inducing conduction abnormalities

Passive model of CHB

A passive model of CHB was developed in BALB/c mice [3]. Timed pregnancies were injected with anti-SSA/Ro –SSB/La autoantibodies purified from human sera from mothers with CHB children. ECG screening of the pups from the injected mothers showed sinus bradycardia and AV block as compared to controls. IgG from mothers with CHB children induced I degree AV block in 88% (14/16), 90% (9/10) and 47% (14/30) in pups injected at 8, 11 or 16 days gestation, respectively [3]. Sinus bradycardia was also present in 44% (7/16), 70% (7/10) and 33% (10/30) of pups injected at 8, 11, or 16 days gestation [3]. Interestingly, no complete AV block was observed.

Active model of CHB

An active model of CHB was created by immunizing female mice with human or murine SSA/Ro 52, SSA/Ro 60 or SSB/La 48 antigens [8,52]. A high incidence of advanced degrees of AV block (II and III) was observed with the immunization using SSA/Ro 52. The average of the incidence of I degree AV block in these models was around 21% which is similar to the incidence reported in humans. However the incidence of III degree AV block was 2.5%. Specifically, in the study by Miranda-Carus et al., the authors used female BALB/c mice that were immunized with human recombinant 48-kDa SSB/La, 60-kDa SSA/Ro, 52-kDa SSA/Ro (52α), and 52 β(amino acids 169–245 deleted) as well as with murine recombinant 52-kDa SSA/Ro. They showed that I degree AV block was detected in 7% of 27 pups born to mothers immunized with 48La, 20% of 54 pups born to 60Ro-immunized mothers, 6% of 56 pups born to 52α immunized mothers, 7% of 86 pups born to 52 β-immunized mothers, and 9% of 22 pups born to mothers immunized with murine 52Ro. Advanced conduction abnormalities were only identified in offspring of 52α- or 52 β-immunized mice. In the 52β group, five pups had complete block [8].

Using a peptide from the SSA/Ro 52 spanning amino acids 200-238 referred to as p200, I degree AV block was induced in 19% (10/52) rat pups [53]. Eftekhari et al. immunized female mice with four peptide from the putative cross reactive sites in the 5-HT4 serotonin receptor. They demonstrated that pups from mothers immunized with 5-HT4 peptides showed bradycardia and AV block (13% of pups) [54]. Immunization with SSA/Ro 52β in Balb/c mice induced AV block in 12% (10/86) in which 5/86 pups had complete III AV block [8]. SSA/Ro 60 and SSB/La 48 induce I degree AV block in 17% (16/97) for SSA/Ro 60 and 7% (4/55) for SSB/La (averaged total from 2 studies Miranda-Crus 1998 and Suzuki 2005 [55]). As suggested from human studies, SSA/Ro 60 and SSB/La 48 seem to be less associated with CHB induction [56,57]. Xiao et al immunized female rabbits with human 52 kDa SSA/Ro and identified that 1 pup (0.7%) had second degree AV block with 2:1 patterns, 7 pups (4.6%) showed sinus bradycardia, 8 pups (5.3%) showed first degree AV block, and 5 pups (3.3%) had both sinus bradycardia and AV block. They also noticed that 31 out of 152 pups (20.4%) were born dead [52].

The results provide strong evidence for the pathogenic role of maternal autoantibodies, particularly anti-SSA/Ro 52 autoantibodies in the development of CHB.

Maternal antibodies induce sinus bradycardia and AV block in Langendorff perfused whole hearts

A question was raised whether direct perfusion of an isolated beating rat heart with maternal IgG would also result in electrocardiographic conduction abnormalities similar to those seen in affected infants. The effects of maternal IgG containing anti-SSA/Ro-SSB/La antibodies on the ECG recording of an isolated rat heart perfused by the Langendorff technique were assessed. Recordings were done using a conventional ECG machine in lead I. After 5 min of perfusion with maternal IgG (800 mg/ml), there was bradycardia associated with 2:1 second-degree AV block that degenerated into complete AV block at about 15 min of perfusion. The QRS complex is absent but the P waves were clearly seen. After 25 min of reperfusion with Tyrode's solution, only partial recovery was seen. In contrast, perfusion of the heart with normal IgG from healthy mothers with healthy children did not alter ECG parameters. The sinus bradycardia and AV block was similarly demonstrated in Langendorff perfused human hearts [4] and by other groups [58–61].

Maternal antibodies specifically inhibit L-type Ca current ($I_{Ca\text{-}L}$) in single cardiac myocytes

The effect of maternal IgG on the L-type Ca current was tested in ventricular cardiomyocytes, SA and AV nodal cells. Maternal antibodies inhibited the $I_{Ca\text{-}L}$ by 62%, 46.2% and 51% in ventricular myocytes, SA and AV nodal cells respectively. The maternal antibodies had no effect on the transient outward K current (I_{to}) [62], the delayed rectifier K current (I_{Ks}) [62] and the fast Na current (I_{Na}) [62] indicating specificity for Ca channels.

Maternal antibodies autoantibodies inhibited Cav1.3 $I_{Ca\text{-}L}$ in native cardiomyocytes

The challenge of investigating the electrophysiological effects of the autoantibodies on individual L-type Ca channels has been difficult because 1) both Cav1.2 and Cav1.3 Ca channels contribute to the total $I_{Ca\text{-}L}$ [63] in the native cardiomyocytes, 2) both are sensitive to the Ca channel blockers and 3) there is no biophysical method to functionally separate the Cav1.2 from Cav1.3 Ca channels. The use of transgenic mouse models has been limited due to embryonic lethality at E14.5 of the Cav1.2 knockout mouse [63]. Therefore, the study and characterization of postnatal Cav1.3 in isolated cardiac cells by Cav1.2 gene deletion is not possible.

As a result, the study of Cav1.3 $I_{Ca\text{-}L}$ has been limited to expression systems such as tsA201 cells which do not express endogenous Ca channels thus allowing for the individual expression of either Cav1.3 $I_{Ca\text{-}L}$ or Cav1.2 $I_{Ca\text{-}L}$. In this regard, we recently reported a novel model of effective lentiviral silencing of Cav1.2 that allowed for the investigation and characterization of the Cav1.3 Ca channels in native cardiomyocytes [64]. The addition of maternal autoantibodies following the silencing of the Cav1.2 gene inhibited the Cav1.3 $I_{Ca\text{-}L}$ by 35%. This observation provided the first evidence that the Cav1.3 $I_{Ca\text{-}L}$ is inhibited in native cardiomyocytes.

Maternal antibodies directly recognize the L-type Ca channel proteins (Cav1.2 and Cva1.3)

To demonstrate that IgG from mothers with CHB children binds directly to the L-type Ca channels α1C and α1D protein, Western blot following immunoprecipitation was used. Immunoprecipitated α1C from human fetal hearts was probed using commercial anti-α1C

antibody and IgG. Both recognized the same molecular weight band. This effect was not observed with IgG lacking the anti-SSA/Ro –SSB/La antibodies [52]. Similarly, cross-reactivity of maternal antibodies with L-type Ca channel α1D protein was detected. Western blot experiments were performed on proteins extract with IgG and anti- α1D antibody. Anti-α1D antibody recognized the 190-kDa band corresponding to the α1D Ca channel protein. IgG from mothers with CHB children but not healthy mothers recognized the same 190-kDa α1D Ca channel protein band.

To further dissect the site of action of maternal antibodies on the α1D subunit, we generated GST fusion proteins corresponding to the extracellular loop S5–S6 of each of the four domains that form the pore of the Ca channel α1D subunit and tested their reactivity with sera from mothers who have children with CHB. The results demonstrated that a fraction (14.4%) of maternal sera whose children have CHB reacted specifically with the extracellular loop of S5–S6 of the first, but not the second, third or fourth domain of the α1D subunit as demonstrated by both ELISA and Western blots [65]. Furthermore, the ELISA positive sera inhibited the expressed α1D Ca current. We concluded that the mere presence of anti-α1D Ca channel antibodies in the sera of mothers with CHB children suggest that additional risk factors may contribute to the pathogenesis of CHB.

Possible reversal of electrocardiographic abnormalities by upregulation of Ca channels

Recently, we hypothesize that if inhibition of the Ca current is critical in cardiac conduction disorders found in CHB patients, then upregulation of the Ca current should rescue or reverse the electrocardiographic abnormalities seen in CHB. The cardiac specific overexpression of the Cav1.2 α1C subunit of the L-type Ca channel was achieved. Detailed molecular, hemodynamic and electrophysiological characteristics of these transgenic (TG) mice are reported elsewhere [66]. Briefly, the Cav1.2 α1C transcript was increased by 2.8 fold. Similarly, the density of $I_{Ca\text{-}L}$ increased by 44% to 52% in cardiac myocytes from the TG mice. This percent increase is ideal since maternal autoantibodies inhibit $I_{Ca\text{-}L}$ within the same range, 40–60% [10,62]. Therefore, we postulated that immunization of TG mice overexpressing Cav1.2 should give birth to pups with no or fewer electrocardiographic abnormalities. Indeed, a lesser degree of sinus bradycardia and fewer AV conduction abnormalities were observed in TG pups from immunized mothers overexpressing Cav1.2 (unpublished data). The finding that TG pups from Cav1.2 overexpression mothers had reduced conduction abnormalities following immunization with SSA/Ro and SSB/La, points to the importance of finding a suitable Ca channel agonist that could help infants diagnosed with CHB and ameliorate the severity of conduction abnormalities. The therapeutic implications can be directed towards the restoration of Ca channel function by the use of L-type Ca channel agonists which will only enhance the fetal, not the mother's Ca channels. In this regard, we have recently shown that Bay K8644, an L-type Ca channel agonist, was not only able to reverse the inhibitory effect of maternal antibodies of Ca current but restored it above the basal level [10]. Efforts are being directed now towards the development of L-type Ca channel agonists which will only enhance the fetal Ca channels.

All together, the Ca channel hypothesis provides evidence supporting an etiologic role of maternal antibodies involvement in the pathogenesis of CHB and point to an essential role of Ca channels in the functional development of CHB.

Heart failure

It is interesting that around 10% of children with CHB not only exhibit conduction abnormalities but also mechanical failure leading to heart failure likely due to inhibition of

the ventricular Ca channels. Cav1.2 is the major channel involved in the EC coupling. However, Cav1.3, which is absent in the adult ventricle, is expressed in the ventricles during fetal stage. It has been suggested that the presence of the Cav1.3 Ca channel in the ventricles during fetal life is needed to aid in the EC coupling since fetal cardiomyocytes lacks the normal Ca induced Ca release (CICR) found in the adult heart. CICR plays a critical role in cardiac contractile function as it is a major source of Ca. However, there are striking morphological and functional differences between the mature and developing heart sarcoplasmic reticulum (SR). In contrast to the adult heart, the SR is sparse and less functional during fetal life. It has been proposed that as a consequence of the underdeveloped SR, the sarcolemmal Ca channels play a major role for Ca delivery to the contractile proteins in the fetal heart. Therefore, the presence of both Cav1.2 and Cav1.3 during fetal stages is necessary to compensate for the lack of a developed SR in order to trigger the contractile function of the myocardium. Conversely, blockade of the Ca channels in the fetal heart by maternal antibodies will diminish the contractile force and worsen cardiac function eventually leading to heart failure even in the presence of a pacemaker.

Proposed Hypothesis for the Pathogenesis of CHB

Two distinct consequences of Ca channel blockade by maternal antibodies can be identified and are summarized in Figure 1: Acute (minutes) and chronic (weeks) effects. We proposed that the *in utero* consequences of this *chronic* inhibition of $I_{Ca\text{-}L}$ could lead to the following scenario: Fetal heart Ca channels are *chronically* exposed to maternal antibodies during pregnancy (starting at about the 12^{th} week of gestation when significant amounts of IgGs are detected). Our hypothesis is that this chronic exposure of Ca channels to maternal antibodies could lead to internalization, degradation and eventually to cell death since Ca channels, as pointed earlier, have been reported to play a vital role in fetal EC coupling. This is consistent with the finding that cross-linking of adjacent ion channels by the two F_{ab} arms of IgG increases the rate of normal internalization of the target protein/antibody complex and thereby decreases the channel density on the cell surface [67]. Cells from fetuses whose Ca channel density is too low (step 4) will eventually die and those whose Ca channel density, although reduced (step 3 and 3a), but still provides the necessary Ca entry for cell function will survive (3a) and will allow AV conduction to occur. This scenario could account for the discordance in twins. Ca dysregulation/apoptosis (5) could favor the translocation of Ro/La to the membrane where they are accessible to maternal antibodies (6) leading to inflammation, cell death, fibrosis and calcification. All together, autoantibodies and Ca channels are causally related to the development of CHB but the range and frequency of conduction defects suggest that additional factors may be necessary to explain the full spectrum of CHB.

Future directions

During the recent few years, significant advances have been made in the understanding of the pathogenesis of CHB. The limitations associated with each of the above hypotheses to explain the full spectrum of CHB will likely guide the future efforts in the search for a unifying hypothesis for CHB pathophysiology. Indeed none of the above mentioned mechanisms can yet account for the low incidence of CHB in infants from mothers with Ro/La antibodies and for the vulnerability of the fetal, but not the mother's heart to CHB. Identification of the extracellular loop of domain I S5-S6 of L-type Ca channel Cav1.3 subunit as a target for autoantibodies from mothers with CHB children will provide novel insights into the development of therapeutic peptides that could bind to the pathogenic antibodies, thereby, preventing the initiation and progression of CHB. The finding that Cav1.2 overexpression mice reduced the conduction abnormalities following immunization with SSA/Ro and SSB/LA, points to the importance of finding a suitable

Ca channel agonist that could help infants diagnosed with CHB and reverse the severity of conduction abnormalities.

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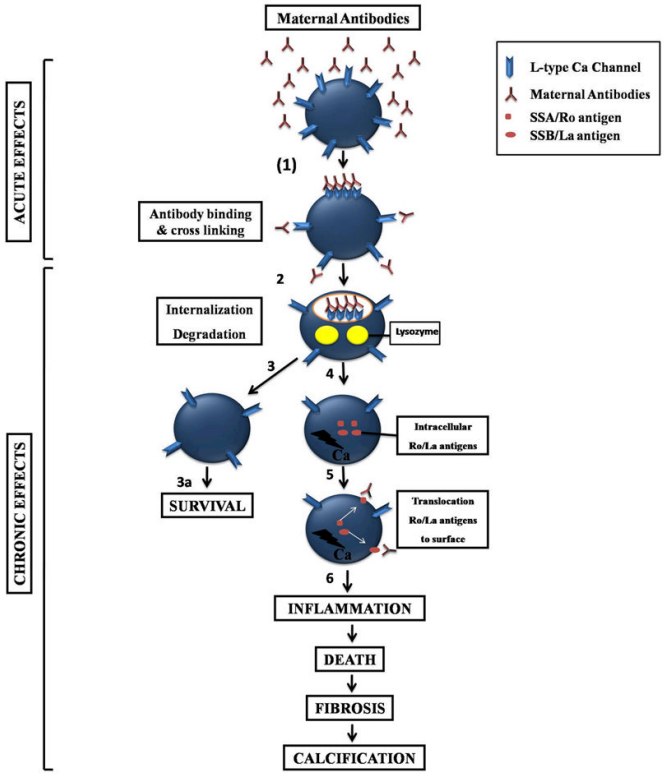

Figure 1.

Schematic representation of maternal antibodies interaction with cardiac Ca channels. The acute events are initiated by binding of the circulating maternal antibodies to surface L-type Ca channels. This leads to cross-linking the antibody-antigen complexes (step 1). As a result of the interaction, the surface complex is internalized and degraded by lysozymes (step 2). In step 3 and 3a, two scenarios are proposed: fetal cells with enough remaining Ca channels will survive (fetal discordance of twins) and secure atrioventricular conduction; those with severe depletion of Ca channels (step 4) will result in Ca dysregulation and apoptosis (step 5). This may lead to the translocation of the intracellular SSA/Ro and SSB/La to the cell surface to bind to their cognate antibodies (step 5). Altogether, this process will eventually trigger inflammation (step 6), cell death, fibrosis and calcification (see text for more details).