Workshop report

Fourth International Workshop on Neonatal Lupus Syndromes and the Ro/SSA-La/SSB System

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Key words: neonatal lupus, fetal heart block, congenital heart block, anti-Ro, anti-La, anti-SSA, anti-SSB.

Introduction

The fourth international meeting on neonatal lupus syndromes and congenital heart block, organized by A. Brucato, F. Franceschini and B. Canesi, was held on May 27-29, 1998 in Milan, Italy. During this interdisciplinary workshop rheumatologists, immunologists and dermatologists expert in these fields met with cardiologists, obstetricians, pediatricians and pathologists to present and discuss the latest data concerning the Ro/SSA and La/SSB system and its relationship to neonatal lupus.

The Ro/SSA-La/SSB System

The Ro and La autoantigens are ubiquitous in all nucleated cells. Human Ro RNPs consist of a 60 kD protein (Ro60) and one RNA of the Y family (hY1, 3, 4 and 5). The 48 kD La protein is also stably associated with a fraction of the Ro RNPs. Other proteins such as Ro52 and calreticulin have also been proposed to be associated with the Ro RNPs, but the exact nature of their physical interactions remains controversial.

The major antigenic component is a polypeptide of 60 kD. Ro60 contains an RNA-binding protein consensus motif which could account for its direct interaction with small cytoplasmic hY-RNAs. It has been suggested that Ro60 may function as part of a novel quality control or discard pathway during 5S rRNA production.

Another target of the anti-Ro autoimmune response is the 52 kD protein (Ro52). The full length protein, 52 alpha, has three distinct domains: an N-terminal region rich in cysteine/histidine motifs and containing two distinct zinc fingers known as RING fingers and B-box; a central region containing two coils with heptad periodicity, one being a leucine zipper with the potential for intramolecular dimerization; and a C-terminal *rfp*-like domain. The Ro52 protein has a high degree of homology with the *ret* finger protein *rfp*, which forms part of the transforming gene *ret*; this raises the question as to whether Ro52 might have a similar transforming potential. An alternatively spliced transcript of the full-length 52 kD Ro, 52 beta, has been recently identified in which exon 4 encoding aa 168-245, inclusive of the leucine zipper and an immunodominant epitope, has been deleted. The involvement of this newly described Ro 52 beta in the pathogenesis of congenital heart block (CHB) has been recently proposed.

Anti-La antibodies recognize a 48 kD polypeptide that does not share antigenic determinants with either Ro52 or Ro60. The La polypeptide is comprised of at least two structural domains on the native protein, each of which contains a distinct antigenic binding site. La facilitates the maturation of RNA polymerase III transcripts, directly binds a spectrum of RNAs, and associates at least transiently with 60 kD Ro.

The autoantibody response to Ro and La is complex. The antigenicity of the Ro antigen is conformation-dependent, and is often not detected when the antigen is fixed *in situ* (e.g., in a cell line for the antinuclear antibody test) or by other methods [e.g., by boiling in sodium dodecyl sulfate (SDS) for the Western blot test]. In fact, the Ro antigen as expressed by bacterial systems shows a similar profound reduction in antigenicity. Thus, Western blotting or the use of an Ro antigen-utilizing expression in a bacterial system from recombinant cDNA can provide misleading results unless precautions are taken. The detection of anti-La presents fewer technical problems for two reasons. First, the concentration of La in the cell is at least 10-fold higher

than that of Ro, and secondly the antigenicity of La is far less conformation-dependent. Antibodies to an endoplasmic reticulum protein called calreticulin have also been described in association with the anti-Ro response, and antibodies to a 57 kD protein have recently been identified as well.

The 60 kD Ro, 52 kD Ro and 48 kD La proteins have been cloned and sequenced. The 57 kD protein and calreticulin have also been sequenced. None of these proteins show any sequence homology. The reason why autoantibodies to these distinct, non-homologous antigens so frequently co-exist is not completely understood. M. Reichlin has shown a cross-reaction between antibodies to the native 60 kD Ro protein and antibodies to the denatured 52 kD Ro protein. Evidence also exists to suggest the presence of a macromolecular complex of Ro and La, with 60 kD Ro and La binding to hY1 RNA, while the physical association of Ro 52 remains controversial. Furthermore, calreticulin binds to the 52 kD Ro protein *in vitro* and might promote the binding of 60 kD Ro to the hY-RNA molecules by directly binding to hY-RNA.

Basic research and molecular biology related to the Ro/La system

T.P. Gordon (Bedford Park, Australia) (1) described epitope spreading and autoimmunity to Ro and La as a paradigm for understanding the normal mechanisms of B and T cell tolerance and the development of autoimmunity to sequestered autoantigens. The immunization of healthy mice with individual protein components of the La/Ro RNP complex induced the production of autoantibodies which recognized Ro60, Ro52 and La, and in some cases the molecular chaperones calreticulin and the heat shock protein *Grp78*. Diversified anti-La/Ro antibody responses were initiated by challenge with a single subdominant T epitope of La, even though some self-epitopes of La were efficiently tolerised. The pattern of the autoantibody response was strongly influenced by the HLA class II phenotype; thus, the HLA class II alleles may play a critical in the initiation and spreading of systemic autoimmune reactions.

Molecular mimicry of such determinants by exogenous agents might readily initiate the spreading of an autoimmune response in genetically susceptible hosts. The exact mechanism by which this occurs is unknown, but epitope spreading is probably driven by inter-molecular help from Ro/La primed T cells to B cells with autospecificity for La or Ro epitopes, and presupposes a physical association or co-localisation (e.g., apoptotic blebs) of the antigens.

M. Bachmann (Mainz, Germany) presented a survey of the physiological expression of the autoantigen La. A cDNA library from the peripheral blood lymphocytes of a patient with systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS) was screened with patient serum. Five La cDNAs were isolated. In two cases the exon 1 was replaced with an alternative, i.e. exon 1'. At the DNA level two independent promoter regions, a series of putative transcription factor binding sites, and an NFκB element were identified. The expression of either exon 1 or exon 1' La mRNA was based on a promoter switch in combination with alternative splicing. At the RNA level Bachmann found that both mRNAs represented finally processed cytoplasmic mRNAs. At the protein level both mRNAs were translated to La protein. Moreover, he identified an internal ribosomal entry site in the La mRNAs, thus explaining the translation of the unusually structured exon 1' La mRNA.

In a further study Bachmann noticed that one of the La cDNAs contained a frame shift mutation in exon 7; he suggested that this region could represent a hot spot in the human genome. Due to the frame shift mutation, the mutant La peptide carried a foreign amino acid sequence which represented a putative neo-epitope. Screening the human La cDNA library and a rat cDNA library, 2 cDNAs were co-isolated which encoded for an unknown protein with a MW of 180 kD which Bachmann termed LaXp180. Sequence analysis showed it to have sequence homology with the autoantigen La and with the B1 chain of laminin. Notably, the La homology represented the hot spot region. Based on these findings, Bachmann proposed a "neo-epitope" working hypothesis: i.e., that the expression of the mutant La mRNA could induce an immune response to the neo-epitope and to the protein LaXP180 which is expressed in the cells of the parotid and salivary glands.

Novel proteins associated with Ro

E. Chan (La Jolla, California) (2) has identified an autoantibody to a novel 75 kD phosphoprotein associated with the 60 kD Ro autoantigen. Using yeast two-hybrid cloning to screen for proteins associated with the 60 kD Ro, a novel 75 kD protein (pp75) was found to interact with the carboxyl 70% of the 60 kD Ro protein. These findings were confirmed using a mammalian two-hybrid assay. The identified full-length cDNA was 3.3 kb. The open reading frame consisting of 541 amino acids had a relatively high percentage of serine residues (15%). This is consistent with *pp75* being a phosphorylated polypeptide determined in immunoprecipitation using extracts from [^{32}P]-radiolabeled HeLa cells. *pp75* is expressed in all tissues, including the human heart. Rabbit anti-recombinant *pp75* gave predominantly cytoplasmic staining. The complete pp75 cDNA has been subcloned into a eukaryotic expression vector with an N-terminal epitope tag T7. Western blot and ELISA analysis showed that 15 of 82 sera (18%) from mothers of babies with NLE recognized the recombinant protein. This data suggests that *pp75* is a novel autoantigen and may interact with the 60kD Ro protein transiently *in vivo*. Furthermore, Chan described the splicing of the human 60kD Ro gene into the two mRNAs, 60 alpha and 60 beta.

G. Boire (Sherbrooke, Quebec) (3, 4) also described the characterization of a novel protein (RoBPI) that interacts with recombinant RohY5 RNPs in yeast. To detect these proteins he has developed a novel assay, the RNP interaction trap assay (RITA), in which RNPs reconstituted in yeast composed of recombinant Ro60 (rRo60) and hY (rhY) RNAs are used as bait to screen for putative RNP/protein interactions. By this means he has identified a novel protein that interacts only with recombinant Ro RNPs containing hY5 RNA (and not the other hY RNAs). This 60-65 kD protein is distantly homologous to various splicing

factors, is widely distributed in human tissues, and can be co-immunoprecipitated with Ro60 from HeLa cell extracts that have been previously treated with a cross-linker. His group is in the process of characterizing this novel protein. Boire also reviewed data from the literature suggesting that Ro52 is not a Ro-RNP-associated protein. To date, biochemical purification of the Ro RNPs has repeatedly failed to show any association with Ro52. More importantly, inhibition of anti-Ro 60 reactivity in anti-Ro sera leaves the anti-Ro52 antibodies unaffected, but these anti-Ro52 antibodies are unable to immunoprecipitate Ro RNPs. Boire currently believes that Ro-52 is not a Ro RNP-associated protein.

Anti-Ro antibodies

M.B. Frank (Oklahoma City, Oklahoma) described the spectrum of the autoimmune response to the Ro52 protein. Relatively few studies have searched for anti-Ro52 in diseases other than SLE and SS. Frank has confirmed Rutjes' findings (5) in patients with idiopathic inflammatory myopathies, showing that antibodies to Ro52 occur in 70% of sera from patients with anti Jo-1 antibodies, but that they are also present with similar frequencies in sera containing antibodies to two other aminoacyl-tRNA synthetases (threonyl- and alanyl-tRNA synthetase) linked to the "anti-synthetase syndrome" (polymyositis, interstitial lung disease, arthritis). Antibodies to Ro52 were also found in 50% of the sera containing anti-SRP (signal recognition particles) or anti-PM-Scl antibodies. However, antibodies to Ro52 were absent in sera containing anti-Mi2 antibodies, which are primarily produced by patients with dermatomyositis. Anti-Ro52 antibodies were also found in only a few sera from anti-topoisomerase I (Scl-70) or anti-centromere positive scleroderma patients, and in only 1 of 41 sera from rheumatoid arthritis patients. The absence of antibodies to Ro60 and La in the sera of these patients with different autoimmune diseases is striking and raises several questions. In particular, does antigenic spreading fail to occur in such cases or does the autoimmune response to Ro52 pre-date the immune response to these other molecules later in life ?

D. McCauliffe (Chapel Hill, North Carolina) (6, 7) showed that conventional Ro serologic assays fail to detect some, if not all, anti-52 kD Ro autoantibodies. In fact, diagnostic laboratories rely primarily on immunoaffinity-purified bovine Ro antigen containing 60 kD but no detectable 52 kD Ro protein. McCauliffe therefore studied sera that were Ro-positive or Ro-negative by conventional ELISA, Western blot, and/or immunoprecipitation assays utilizing recombinant 52 and 60 kD Ro proteins and native La and 60 kD Ro proteins. Three of 18 primary SS sera that were Ro- and La-negative by conventional assays were positive to recombinant 52 kD Ro by ELISA and Western blot and also immunoprecipitated native 52 kD Ro from cell extracts. Ten Ro-negative sera from infants with suspected neonatal lupus based on the presence of cardiac disease were similarly tested by ELISA. Two of these were reactive against the recombinant 52 kD Ro ELISA. McCauliffe concluded that conventional serologic assays based on affinity purified bovine Ro antigen can detect the majority of patients with anti-Ro antibodies. However, these methods are inadequate to detect anti-Ro antibodies in the small number of patients who have anti-52 kD Ro antibodies in the absence of anti-60 kD Ro antibodies. This failure to detect anti-52 kD Ro antibodies could present serious problems, particularly in SS patients and in the neonatal lupus syndromes.

S. Bombardieri (Pisa, Italy) (8) described recent results from his group showing that anti-Ro antibodies are the most frequent antibodies in patients with "true" undifferentiated connective tissue disease (UCTD), and that these antibodies are correlated with xerostomia and xerophthalmia. Bombardieri concluded that the UCTDs in most patients represent distinct clinical entities with a limited autoimmune repertoire (most often anti-Ro antibodies) rather than the early phase of a definite connective tissue disease. During the ensuing discussion similar results were reported by F. Franceschini (Milan, Italy). In addition, Bombardieri described the case of an anti-Ro-positive mother who had already given birth to a CHB baby. During her next pregnancy she was treated with high dose intravenous immunoglobulins to prevent the recurrence of CHB. Notably, the titres of anti-Ro and anti-La antibodies as detected by ELISA were unaffected by this treatment; nevertheless, the second baby was born without CHB.

Pathogenetic problems

R.H. Scofield (Oklahoma City, Oklahoma) (9) presented recent data showing that anti-Ro mediates granulocytopenia via binding of the D1 protein, a novel antigen in SLE. His current work suggests that anti-Ro is associated with granulocytopenia, binds to the surface of granulocytes, and fixes complement to the membrane surface. Binding is a property of the anti-Ro Fab fragments and is inhibited by 60 kD Ro. However, using a procedure involving affinity purification, trypsin digestion and mass spectroscopy, the antigen bound to the surface of the granulocytes has been identified as a 64,000 MW protein known as D1. This protein is a novel antigen in SLE. As suggested by inhibition studies in which a sequence from the 60 kD Ro shared with D1 was found to block neutrophil binding, sequence identity between 60 kD Ro and eight tandem repeats in the 64 kD D1 antigen may be responsible for the observed serological cross-reaction. In Scofield's opinion, these data imply that the anti-Ro antibodies which bind D1 may mediate neutropenia.

R. Herrera Esparza (Zacatecas, Mexico) presented data concerning the ontogeny of Ro hYRNA in the human heart. Samples of cardiac tissue from embryonic and adult subjects were obtained from legal autopsies and studied by *in situ* hybridization and reverse-transcription PCR assays. Probes for hY1, hY3, hY4, hY5and 5s rRNA were labeled. He demonstrated that: (i) the expression of hYRNAs was greater in embryonic tissues and was enhanced in the embryonic heart; (ii) hY4 and hY5 RNAs were expressed intensely between 8 - 12 weeks of intrauterine development; and (iii) high transcription rates of hY5 were demonstrated at week 12 of gestation. The finding that hYRNAs are mainly expressed during heart development could help to clarify the role of hYRNA

in the pathophysiology of CHB.

P. Venables (London, UK) reviewed the possible role of viruses in CHB (10). Venables, Horsfall *et al.* (11) recently described the role of a human endogenous retrovirus (ERV-3) as a potential new target antigen in the pathogenesis of CHB. ERV-3 is a full-length endogenous pro-virus which is present as a single copy per haploid genome. The *env* protein is abundantly expressed in placental syncytiotrophoblast, thus suggesting that it may have a beneficial biological function for the host. ERV-3 is also expressed in other fetal tissues, most strongly in the fetal heart. Maternal antibodies to ERV-3 are induced during pregnancy, but appear to be tightly regulated since their levels fall post-partum. In women with autoimmune disease, regulation is perturbed so that anti-ERV-3 antibodies may persist post-partum, with the highest titers being associated with mothers of babies affected by CHB. Venables *et al*. have also recently described a novel human retrovirus, human retrovirus 5 (HRV-5) (12), from the salivary gland of a patient with SS.

A.C. Horsfall and E. Neu (London, UK) (13) presented recent data concerning the role of maternal autoantibodies and the placenta in CHB. These authors have previously shown that IgG eluted from the cardiac tissue of a baby with CHB reacted with La, and that a subpopulation of these antibodies cross-reacted *in vitro* with laminin-2 (merosin), a component of the basement membrane of both the heart and placenta. These authors have now studied a patient with SLE/SS during two consecutive pregnancies, 13 months apart, the first resulting in the delivery of a male child with CHB and the second in the delivery of a healthy female child. The maternal titers of anti-La and Ro52 were comparable in the normal and CHB pregnancies. In contrast, the titer of anti-La antibodies in the placenta and cord blood showed striking differences between the two pregnancies; only during the CHB pregnancy was anti-La activity depleted, by 30% in the cord blood and by 63% in the placental eluates compared to the activity in the maternal serum, a finding consistent with the deposition of anti-La antibodies in the fetal tissues. Moreover, the titers of antibodies to Ro60 and ERV-3 were high in the CHB pregnancy, but were almost negative in the healthy pregnancy. Furthermore, the specific activity of anti-ERV-3 was enriched by more than 20-fold in the placenta, suggesting specific binding to the syncytiotrophoblast antigen, while a modest depletion was found in the cord blood. In conclusion, the specific depletion of antibodies to ERV-3 and La in the cord blood of the affected child suggests that these two specificities may contribute to the pathogenesis of CHB.

M. Reichlin (Oklahoma City, Oklahoma) recently studied the acid-elutable anti-Ro content in various tissues from a child who died of complete heart block. Here he compared his results with those of a similar study conducted in 1994 (14). In the present case there was a five-fold (500%) reduction in the IgG content of the cord blood relative to the maternal sample. In the previous case, the anti-Ro activity per µg of IgG in the acid eluates of the heart were enriched three-fold over the anti-Ro activity in the cord blood. In the present case, the yields of acid-elutable IgG per gram of tissue were much greater than in the previous case (3 - 4.0 µg/g for the brain, skin, and kidney eluates, and 15.7 µg/g for the heart eluates). While there were massive deposits of IgG in the tissues, especially the heart, there was no enrichment of anti-Ro in any organ. Reichlin also described another study of anti-P (anti-ribosomal P) peptides in neonatal lupus, which appeared to show that there is no increase in the prevalence of anti-P antibodies in CHB mothers.

J. Buyon (New York, NY) (15) first discussed apoptosis and CHB, showing results regarding the accessibility of Ro and La antigens to maternal autoantibodies in apoptotic human fetal cardiac myocytes. The cellular topologies of Ro and La were evaluated by indirect immunofluorescence using anti-sera (one a monospecific anti-La, and the other recognizing both 52 and 60 kD Ro). In non-apoptotic cardiocytes, Ro was predominantly nuclear with little cytoplasmic staining while La was confined to the nucleus. In early apoptotic cardiocytes the condensation of Ro- or La-stained nuclei was observed, accompanied in some cases by a rim of green fluorescence around the cell's periphery. In the later stages of apoptosis the nuclear Ro and La staining became weaker. Propidium iodide (PI) demonstrated nuclear fragmentation. Ro/La stained blebs emerged from the cell membrane (a finding also observable in non-permeabilized cells), thus supporting the hypothesis of an antibody-antigen interaction at the cell surface.

Thus, the induction of apoptosis in cultured human fetal cardiac myocytes results in the translocation of Ro and La to the cell surface, which could facilitate its recognition by circulating maternal antibodies. Although apoptotic cells are already programmed to die and, unlike necrotic cells, do not characteristically evoke an inflammatory response, the "binding" of maternal antibodies and subsequent influx of leukocytes could result in damage to surrounding healthy fetal cardiocytes.

Buyon also described her recent experiments on the induction of antibodies reactive with Ro-La and the development of CHB in a murine model (16). Female BALB/c mice were immunized with human recombinant 48 kD La, 60 kD Ro, 52 kD Ro (52 alpha full-length), and 52 beta (aa169-245 deleted), as well as murine recombinant 52 kD Ro. First degree block was detected in 7% of 27 pups born to mothers immunized with 48 kD La, in 20% of 54 pups born to 60 kD Ro-immunized mothers, in 6% of 56 pups born to 52 alpha-immunized mothers, in 7% of 86 pups born to 52 beta-immunized mothers, and in 9% of 22 pups born to mothers immunized with murine 52 kD Ro. More advanced conduction abnormalities were only identified in the offspring of 52 alpha- or 52 beta-immunized mice. Specifically, in the 52 alpha group one pup had a complete block while another had a second degree block; in the 52 beta group 5 had a complete block. Maternal antibodies to the primary immunogens were detected in the pups. None of the controls exhibited any conduction abnormalities. This antibody-specific animal model provides strong evidence for a pathogenetic role of the anti-Ro/La antibodies, particularly 52 kD Ro, in the development of CHB. The range and frequency

of conduction defects found suggest that additional factors may also promote disease expression.

L. Rossi (Milan, Italy) discussed the pathology of CHB in the light of his findings in three anti-Ro positive patients and one anti-Ro and anti-La negative patient. The pathology of the 3 anti-Ro positive cases was similar to cases already reported in the literature. Endocardial fibroelastosis was present and the AV node was replaced by fibrous tissue. Scattered areas of what used to be assumed was calcified tissue, but which may actually represent hematoxylin bodies made up of deposits of immunoglobulins (17), could be seen. Inflammatory mononuclear infiltrates were rarely present.

In contrast, the pathology of the anti-Ro and La negative case was completely different from the 3 previous cases. The AV node was normal, but a scanty mononuclear infiltrate could be seen contiguous to the AV node. Exceptionally, in this case the heart block reversed to a sinus rhythm after birth. Moreover, for the immunohistochemical recognition of apoptotic cells Rossi used the TUNEL method, which identifies early DNA fragmentation in the nucleus based on the specific binding of terminal deoxynucleotide transferase to the 3'-OH ends of DNA. Scanty apoptotic cells were observed in the area of the conduction tissues in the anti-Ro positive CHB patient.

Clinical findings

W.L. Weston (Denver, Colorado) (18, 19) opened the session of clinical lectures by reviewing the clinical spectrum of cutaneous neonatal lupus. He described the results of a study on 4 boys and 14 girls. Characteristic oval or round red macules with fine scales resembling subacute cutaneous lupus were observed. The lesions were present at birth in 16% of the cases and in the remainder they appeared within 2 months after birth. Photosensitivity was frequent (12/18). The distribution of the skin lesions in 18 infants was as follows: on the face in 17 patients; periorbital "owl-eye" or "eye mask" facial rash in 14 patients; on the scalp in 15 patients; on the arms and legs in 13 patients; and on the trunk and groin in 6 patients. Crusted lesions were predominant in 3, and features of cutis marmorata telangectasia congenita were observed in 4. In 17 babies neonatal lupus was not suspected until the dermatological examination.

Non-cutaneous manifestations included thrombocytopenia in 4, cholestatic hepatitis in 3 and CHB in 3 patients. Four had residual telangectasia which persisted for 3 or more years, but which eventually resolved in 2 cases. Three babies had dyspigmentation that spontaneously cleared within 22 months. None had atrophy or scarring. Where necessary, patients could be treated for hyperbilirubinemia by irradiation with blue fluorescent tubes, as the spectral output is almost entirely in the visible light range. Weston concluded that children with cutaneous NLE should be evaluated for hematologic and hepatic as well as cardiac involvement.

L.A. Lee (Denver, Colorado) (18, 19) described some potential new features of neonatal lupus liver disease. Neonatal cholestasis is a common presenting feature of liver disease in infancy. Most cases can be attributed to idiopathic neonatal hepatitis/idiopathic neonatal cholestasis (INC) or extra-hepatic biliary atresia (EHBA), each of which occurs in approximately 1 of 10,000 live births. The etiologies of these conditions remain obscure. One identifiable cause of neonatal cholestatic liver disease is neonatal lupus erythematosus (NLE). Lee suggested that NLE liver disease can occur as an isolated finding and is currently examining the hypothesis that some cases of INC and/or EHBA may actually be cases of NLE. Preliminary data (11 INC sera, 20 EHBA sera, and 8 control sera) suggest that maternal sera from INC patients may have a higher than expected frequency of low titer antibodies to 60 kD Ro on ELISA, but not on immunodiffusion. Extending this study to a larger number of patients should help to prove or disprove the hypothesis that at least some cases of INC are autoimmune in origin and possibly linked to NLE.

J. Buyon (20) then reviewed the most recent data on demographics, mortality, morbidity and recurrence rates obtainable from a National Research Registry for Neonatal Lupus. This American cohort currently includes 105 anti-Ro positive mothers and their 113 infants diagnosed with CHB between 1970 and 1997 (56 males, 57 females). Bradyarrhythmia confirmed to be due to CHB was initially detected before 30 weeks of gestation in 82% of the cases (median gestation time 23 weeks). No severe structural abnormalities in the heart were found concomitant with the CHB. Twenty-two (19%) of the 113 children died, 16/22 (73%) within 3 months after birth. The cumulative probability of 3-year survival was 79%. Sixty-seven (63%) of 107 live-born children required pacemakers: 35 within 9 days of life, 15 within one year, and 17 after one year. Forty-nine of the mothers had subsequent pregnancies: 8 (16%) gave birth to another infant with CHB and 3 (6%) to a child with an isolated rash consistent with NLE.

Regarding the effects of therapy with dexamethasone, a retrospective assessment showed it to be efficacious against hydrops. As far as AV block is concerned, in Buyon's experience dexamethasone may have some effect in less severe situations. Cases of third-degree block never reverted, but among 4 cases of second degree block treated with dexamethasone reversion to a first degree block was seen in 2; in one case the block reverted but in an unstable manner, fluctuating between a first and a second degree block; while in the fourth case the block remained stable, without ever evolving into a complete third degree block.

H. Julkunen (Vantaa, Finland) (21) presented other new data on CHB. He showed that the long-term outcome in mothers is generally good, since most only develop subclinical primary SS. A low risk maternal profile is characterized by low levels of antibodies on ELISA and a negative immunoblot, while a high risk profile is marked by high levels of antibodies on ELISA and a positive immunoblot showing the presence of anti-52 kD Ro. These results are in agreement with previous observations by Buyon. In Finland 62% of mothers with CHB children have been found to be B8/DR3/DQ2 positive, while the risk of a recurrence of CHB in a subsequent pregnancy is 11.8% (4/34). 5). Mothers of CHB children were not found to have an increased risk of other obstetric complications. All of the tested mothers were

negative for anti-cardiolipin antibodies. High dose iv gammaglobulins and corticosteroids were ineffective in preventing the recurrence of CHB. A nationwide epidemiological study of CHB children born in Finland between 1970 and 1995 showed an incidence of one case of CHB per 11,000 live-born children. The frequency of CHB seems to be increasing since 20 years ago its prevalence was only 1/20,000 in Finland.

A. Jonzon (Uppsala, Sweden) (22) described the natural history of CHB, refuting the previously held but incorrect notion that patients with CHB who are symptom-free have a good prognosis with a low risk of sudden death. He reported the results of a study of 102 patients fulfilling the Yater criteria for CHB (see below), recruited from 1964 onwards. Only those patients who were free from symptoms at the age of 15 years were included. Among these, 50% had CHB diagnosed *in utero* or in the neonatal period. The mean age at follow-up was 38 years (range 6 - 66 years). In this series there were 11 deaths, 5 without any preceding symptoms. Other patients eventually developed symptoms: 19 had non-fatal syncopes, and 8 had repeated fainting episodes. Clinical findings associated with CHB included chest pain, fatigue, nightmares, anxiety, mitral regurgitation and a prolonged QTc. The ventricular rate tended to decrease with age. Surprisingly, in 6 patients AV conductivity improved to a first or second degree block. Slightly more than 50% of the patients had a pacemaker (PM) implanted, which reduced the risk of death. The only statistically significant prognostic sign for death or the development of CHB symptoms was a prolongation of the QTc. Jonzon therefore concluded that there is a risk for sudden death at any age, even in the absence of preceding signs or symptoms. He suggested that the early implantation of a pacemaker could be the treatment of choice for all patients with CHB.

R. Cimaz (Milan, Italy) described his follow-up of 10 anti-Ro positive newborns without CHB. It was observed that these antibodies disappeared after the age of 6 to 8 months. No perinatal complications were seen. One infant presented transient sinus bradycardia in his third day of life, while another showed a prolonged decrease in tear production. In one infant an ECG taken at 3 months showed a prolonged QTc interval (460 msec), which was confirmed by a 24-hour ECG recording. Treatment with propranolol was begun, which resulted in a rapid decrease in the QTc value. The other ECGs of anti-Ro positive infants were then retrospectively analyzed and the mean QTc value was found to be 426 msec ± 21.7. In a large group of normal newborns the mean QTc was 400 msec, with 430 msec representing the 95th centile. Since a prolonged QT interval may be a risk factor for sudden death in the first year of life, Cimaz's findings definitely warrant further study.

J. Stein (Graz, Austria) (23) described the research protocol for the study of fetuses with complete heart block that was developed by the Fetal Cardiology Working Party of the Association of European Pediatric Cardiologists (Chairman: L.D. Allan). The cases for study may be divided into three groups. Group 1 should be comprised of mothers with known anti-Ro antibody whose fetuses do not have CHB; group 2 should comprise the mothers of fetuses with isolated CHB; and group 3 should comprise the mothers of fetuses with CHB in the setting of congenital heart disease. The working party then proposed the following protocol. The mothers in group 1 should be randomized to either steroid therapy (dexamethasone or betamethasone 4 mg/day) or no treatment. Group 2 mothers should be divided into 2 subgroups: (2a) those with non-hydropic fetuses receiving either no treatment or steroids, and (2b) those with hydropic fetuses randomized to one of 3 treatment regimens - steroids, or chronotropes (oral salbutamol 40 mg/day or terbutaline 10-20 mg/day), or no treatment. Group 3 mothers should be separated into 2 subgroups: (3a) those with non-hydropic fetuses receiving no treatment, and (3b) those with hydropic fetuses receiving either no treatment or chronotropes. The mothers must be tested for anti-Ro antibodies; they should also be examined monthly and undergo a standard fetal echocardiogram. This protocol proposes the randomization of dexamethasone to anti-Ro positive mothers who have had no previous fetuses with complete heart block in order to gather as much information as possible regarding risk. This somewhat controversial suggestion stimulated considerable commment and debate among the rheumatologists attending the meeting.

A. Brucato (Milan, Italy) (24) concluded the meeting with a proposal to establish a new definition of CHB. Cardiologists define a block as being "congenital" based on the classical criteria of Yater (1929): "Heart block established in a young patient. There must be some evidence of the existence of the slow pulse at a fairly early age and absence of a history of any infection which might cause the condition after birth: notably diptheria, rheumatic fever, chorea and congenital syphilis". Reliance upon this rather broad definition can lead to situations such as that of J.M. Reid who considered as congenital a case first presenting with symptoms at the age of 85 years (25) ! Furthermore, a complete heart block detected *in utero* will differ from a block detected later in life in at least three important ways: fetal cases have an immuno-mediated pathogenesis, a worse prognosis, and a much higher risk of recurrence in subsequent pregnancies. Ovelaps may occur, of course, but they should be easily detectable since most mothers remain anti-Ro positive even years after delivery. These considerations are of critical importance for the counseling of affected families.

An article is currently under preparation by a small group of internationally known cardiologists, together with rheumatologists and obstetricians (all participants in this meeting), in which an updated definition of congenital heart block will be formally proposed, i.e. a block may be considered as congenital if it is "existing at or dating from birth".

Acknowledgments

The authors gratefully acknowledge funding received from Niguarda Hospital for this meeting. They also would like to express thanks to B. Canesi and F. Franceschini for their invaluable contribution to the organisation and running of the meeting, and to F. Brucato, E. Coluccio, G. Guareschi, S. Pittau, G. Valmadre, and L. Balossi for their kind collaboration.

The complete proceedings of this meeting may be requested from Dr. A. Brucato.

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