Anti-Ro Antibody and Its Significance

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Anti-Ro/SSA antibodies are specific antinuclear antibodies that have been detected in high frequencies in patients with primary Sjögren's syndrome and in systemic lupus erythromatosus (SLE).[1] They are produced by activated B lymphocyte and directed against two major proteins (52 and 60 kDa) which are bound intracellularly as a complex to small ribonucleic acids called hY1, hY3, hY4, and hY5.[2-5] Many reports showed that anti-Ro52 antibodies have a strong association with many conditions, such as primary Sjögren's syndrome, congenital complete heart block (CCHB), certain varieties of SLE, systemic sclerosis and dermatomyositis/polymyositis (PM/DM), while anti-Ro60 antibody is more strongly linked to SLE.[6-11] Anti-Ro/SSA antibodies can be detected by several methods, for example, double immunodiffusion (DID) or Ouchterlony method, enzyme immunoassay (EIA), immunoprecipitation, counterimmunoelectrophoresis (CIEP) and immunoblots or Western blots.[12] These methods have different benefits from one to another and they are used to observe the nature of these antibodies.[13-14] While DID, the standard immunoassay, has been used to detect the precipitin line caused by antigen-antibody interaction, ELISA and immunoblots have been used increasingly because of their advantages in the detection of anti-Ro antibodies.[15-18] Physicians need to know the clinical importance of anti-Ro antibodies; also directors of the laboratory have to understand what type of assay that is suitable for the detection of these antibodies.

Over forty years ago, anti-Ro/SSA antibodies were first described as a precipitin in a patient with Sjögren's syndrome.[19] These heterogeneous autoantibodies are one of specific antinuclear antibodies (ANA) that have been detected in a number of human systemic autoimmune disorders such as primary Sjögren's syndrome, subacute cutaneous lupus erythematosus, neonatal lupus erythematosus, antinuclear antibody-negative lupus erythematosus and systemic lupus erythematosus-like disease secondary to homozygous C2 or C4 complement deficiency.[20-25] They are also related to other disorders, for example, congenital complete heart block (CCHB), systemic sclerosis, dermatomyositis/polymyositis (PM/DM), scleroderma, mixed connective tissue disease and rheumatoid arthritis.[8-11,15] Anti-Ro/SSA antibodies are known to recognize several distinct cellular antigens, and there is substantial evidence that they have a major role in the pathogenesis of the diseases.[25]

What is "Ro"?

Ro was a term first used to define a soluble cytoplasmic antigen which formed a unique line of precipitation in DID studies with sera from patients with SLE and Sjögren's syndrome.[26] From the work of Steitz and co-workers, Ro has been further defined as a cytoplasmic hY RNA binding protein which migrates at 60 kD by SDS-PAGE.[27] Ro ribonucleoproteins (Ro RNPs or Y RNPs), the heterogeneous autoantigens that are recognized by anti-Ro/SSA antibodies, are composed of one of four small cytoplasmic RNAs (84-112 nucleotides) termed Y1-Y5 (Y2 is a degraded form of Y1 RNA) and at least two proteins: the 60 kDa protein, Ro60, and the 48-kDa phosphoprotein, La (Fig 1).[28,29] These groups of Ro RNPs are produced by genes that localize on the short arm of human chromosome 19. Both Ro60 and La are in the group of RNA-binding proteins which are characterized by a conserved RNA-binding domain. A third protein of molecular mass 52 kDa (Ro52) has been reported to be associated with Ro RNPs, but no direct binding of this protein to Y RNAs could be demonstrated. It is shown that Ro52 is completely unrelated to Ro60 and La and

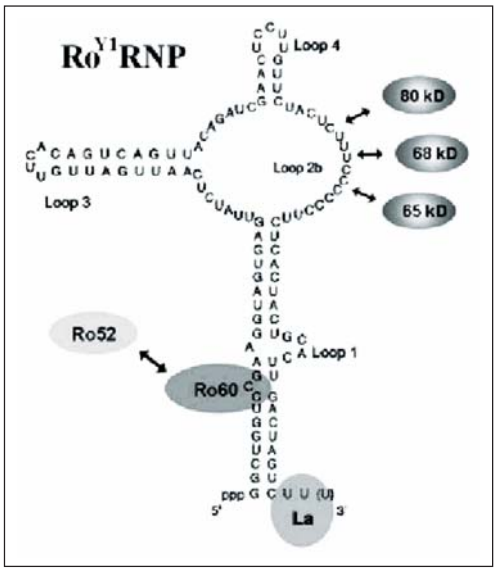

Fig 1. Hypothetical model for human Y1 RNPs. Y RNAs are associated with La and a major portion has the Ro60 protein bound, whereas Ro52 may only transiently interact with (a subgroup of) Ro60 RNPs.

belongs to a group of proteins assumed to be involved in cell activation and transformation, while the roles for La and Ro proteins are in the regulation of eukaryotic translation and regulation of transcription factor.[30] Previous studies demonstrated the biochemical interaction between human autoimmune sera and these Ro RNPs complexes have the ability to categorize patients based on Ro epitope recognition could have great clinical utility if the patient's clinical course and/or response to therapy could be predicted by these results.[4,31]

Correlation of anti-Ro/SSA antibodies to clinical manifestations

Some reports show correlations between subspecificies of anti-Ro/SSA antibodies and clinical manifestations. According to reports on immunoblot data, the anti-Ro52 antibodies have a strong association with primary Sjögren's syndrome, while the anti-Ro60 antibodies are more strongly linked to SLE and secondary Sjögren's syndrome.[6] Other conditions which are also reported to be associated with anti-Ro52 antibodies are CCHB, certain varieties of SLE, systemic sclerosis and PM/DM.

Sjögren's syndrome

In primary Sjögren's syndrome, the prevalence of anti-Ro/SSA antibodies, depending on the assay performed and the population selected, is from 40 to over 95%, so the presence of anti-Ro/SSA antibodies in patients with suspected primary Sjögren's syndrome strongly supports the diagnosis.[3,32] In contrast, the prevalence of anti-Ro/SSA antibodies in secondary Sjögren's syndrome is only 10 to 15%. The association of Sjögren's syndrome and anti-Ro/SSA antibodies has been difficult to explain, since these antigens are found in all nucleated cells. Ro/SSA antigens are found in the bleb of apoptotic cells and do not undergo proteolysis during apoptosis.[33] It is possible that increased expression levels of these antigens during apoptosis or aberrant clearance and processing of antigens derived from dying cells may lead to the accumulation of potentially immunogenic forms of these autoantigens. Thus, high titers of these autoantibodies are usually associated with a greater incidence of clinical manifestrations, especially extraglandular features such as purpura and vasculitis. Alternative mechanisms that might expose or immunize to cryptic epitopes of these autoantigens include structural alterations caused by abnormal protein-protein interactions during aberrant cell death, mutations, and interactions with toxins, chemicals, or foreign antigens derived from microorganism such as viruses.[2] Many studies show that anti-Ro52 antibodies are predominated in primary Sjögren's syndrome and anti-Ro60 antibodies are more frequently linked to secondary Sjögren's syndrome. However, some reports show that the difference of types of antibodies to the diseases is not statistically significant and sera that are reactive to both Ro52 and Ro60 antigens can be seen in about 20.9%[34] and 27%[35] depending on the assays and course of the diseases.

Systemic lupus erythromatosus (SLE)

Anti-Ro/SSA antibodies are found in approximately 24% to 60% of patients with SLE.[34,36] More frequently than in patients with SLE as a whole, anti-Ro/SSA antibodies are detectable in patients with some SLE variants, e.g., with photosensitivity, a rash known as subacute cutaneous lupus (70%-90%), cutaneous vasculitis (i.e. palpable purpura), neonatal lupus and interstitial lupus pneumonitis.[36] In 1% of SLE cases, the detection of ANA is not possible even during the active stages of the disease. However, anti-Ro/SSA antibodies are detectable in the serum in 60% of these patients.[3] When subtypes of anti-Ro/SSA antibodies are considered, it is found that single reactivity towards Ro60 antigen is more predominant than Ro52 antigen in sera of patients with SLE and cutaneous lupus (80% versus 15.8% and 29% versus 9%, respectively) and both reactivity to Ro60 and Ro52 antigens can be found in 52.2% in SLE and 8.7% in cutaneous lupus by line immunoassay method.[34,37] The anti-Ro60 and Ro52 antibodies profile is fixed at an early stage of disease and hardly changed in most patients; patients with fluctuating course of disease tend to have a coordinated expression of these autoantibodies.[38] It is likely that the involvement of anti-Ro60 antibody has more prominent tendency in autologous antibody dependent cellular cytotoxicity than that of anti-Ro52 antibody in the pathogenesis of cutaneous lupus erythromatosus.

Congenital complete heart block (CCHB)

Anti-Ro/SSA antibodies also have a significant diagnostic role during the prenatal monitoring of pregnant women. They are detectable in about 100% of mothers of fetuses or newborns with CCHB or neonatal lupus (photosensitive exanthemas and cytopenias).[9,39,40] These antibodies are considered to be a model example of transplacentally acquired autoimmune phenomena.[8,41] The risk of a mother with anti-Ro/SSA antibodies to give birth to a child with CCHB and neonatal lupus is reported to be 5% to 10%[39]. This risk is considered to be lower if only low anti-Ro/SSA antibody titers are present and not detectable in the immunoblot.[3,9] Anti-Ro antibodies may induce autoimmune injury that prevents normal development of the conduction fibers.[42] It is frequently found that development of CCHB is strongly dependent on a specific profile to Ro52 antigen, which may be a useful diagnostic and monitoring tool to these groups of patients.[7-9] However, depending on the analytic method, recent reports show that antibody to Ro52 antigen is not more specific for or frequent in CCHB than antibody to Ro60 antigen.[43]

Other conditions

Anti-Ro/SSA antibody is one of many antibodies found in systemic sclerosis. It occurs at a lower frequency than in those with SLE or Sjögren's syndrome (less than 35%). However, Sjögren's syndrome has been described in up to 20% of all patients with systemic sclerosis with about one-third to one-half of those with anti-Ro antibodies. Sjögren's syndrome is actually associated with about 35% of systemic sclerosis patients positive for anti-Ro.[44]

There is a study ön the prevalence of 52-kd and 60-kd Ro/SS-A antibodies in Japanese patients with polymyositis/dermatomyositis.[10] The results suggest that Ro52 is the main antigen of anti-Ro/SS-A antibodies in patients with polymyositis/dermatomyositis; and, its coexistence with other defined antibodies implies the existence of a subgroup of patients with various serologic abnormalities.

Detection of anti-Ro/SSA antibodies in the laboratory

Anti-Ro/SSA antibodies can be detected by several methods, for example, double immunodiffusion (DID) or Ouchterlony method, enzyme immunoassay (EIA), immunoprecipitation, counterimmunoelectrophoresis (CIEP) and immunoblots or Western blots. Among these assays, protein immunoprecipitation is the gold standard for the anti-Ro60 antibody detection. Other alternative methods are, namely, RNA immunoprecipitation, enzyme immu-

noassay and DID. In the first time, Western blot, although useful for defining fine specificities, is of limited value for detecting antibodies against conformational epitopes, including Ro60 kDa antigen. However, there are some reports about the adjustment of agarose gel constituent and the use of detergents to improve the test performance by renaturing this protein.[45] So this method can be used to detect Ro60 antigen and it is now commercially available in a form of a kit.

On the other hand, Western blots are the gold standard for detecting anti-Ro52 antibody because the antigenic determinant of this antigen can survive denaturation during the process. Another acceptable method is enzyme immunoassay (EIA) which the recombinant protein is used to increase the test performance. Anti-Ro52 antibody can immunoprecipitate the protein, but they are not reliably detected by that means, and many sera immunoprecipitate Ro60 but not Ro52 antigen.

CONCLUSION

Anti-Ro/SSA antibody is a specific antinuclear antibody that has been detected in many conditions such as primary Sjögren's syndrome, systemic lupus erythromatosus (SLE) and its variants, congenital complete heart block (CCHB), systemic sclerosis and dermatomyositis/polymyositis (PM/DM). The significance of the antibody to these diseases varies according to the nature of the diseases and the antibody. To detect this antibody, many laboratory methods are used. While protein immunoprecipitation is the most reliable method for detection of anti-Ro60 antibody, Western blots are the gold standard for anti-Ro52 antibody detection. It is important to both physicians and laboratory directors to know about the appropriate use of this test in order to choose the best assay to make the most efficacies in the diagnosis of the related diseases.

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