

Review Article

Ro-autoantibody System and Characterisation of Protein Isoforms of Ro60 in Systemic Erythematosus Lupus (An Update)

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Abstract: Systemic lupus erythematosus (SLE) is a multisystem inflammatory and autoimmune disorder that usually affects various self-tissues of the body, whose sera is predominantly reported to have autoantibodies against Ro60 or TROVE-2 protein. Ro60 is a ring-shaped RNA-binding protein, that usually binds misfolded non-coding RNAs, pre-5S rRNA, and several small cytoplasmic RNA molecules known as Y RNAs (hY-RNAs). Y RNAs are known to be involved in regulating cellular stress responses and also in initiation of chromosomal replication. Ro60 is known to have 6 isoforms along with the short isoform. Recent studies of Ro60 protein in mammalian cells suggests that Ro60 is vital for the cell survival after the UV irradiation. It is evident that Ro60 is essential for degrading the damaged RNA due to the UV irradiation, because exposure to the UV irradiation might result in RNA: RNA and RNA: Protein crosslinks. Also, role of Ro60 in maintaining the tolerance is supported by the experiment which resulted in development of lupus like syndrome in the Ro60 knock-down mice by producing antibodies against chromatin and ribosomes. Thus, it is evident from the various studies that Ro60 is inevitably important for the cells and tissues for preventing the autoimmunity. This review focusses on the pathology and autoantibody system in SLE, structure and functions of the Ro60 in association with Y RNAs, and epitope bindings of Ro60 to the anti-Ro positive sera from SLE patients.

Keywords: Self-tolerance, Systemic Lupus Erythematosus, Ro60 Protein, Y RNAs, Anti-Ro Autoantibodies

1. Immunity and Autoimmunity

1.1. Overview of Immune System

The concept of immunity came into existence with the scenario of plague infection in Athens in 430 BC, where the writings of a great historian stating “only those who had recovered from the plague could nurse the sick” gave an initial insight into the topic of immunity, by following a notion that the recovered ones does not develop the disease a second time [1]. Gradually the concept of immunity came into the limelight with the experiments of an English physician Edward Jenner, who tried to develop the immunity in the people against a dreadful disease called Small Pox [1]. Gradual understanding of immune system in detail made it

one of the important and fascinating biological aspects that which scientists had to focus upon. Smith and Germolec [2] defined that Immune system is a complex network which functions by the collaborative work of various cellular, chemical and soluble protein components designed majorly to protect the body against the foreign substances by avoiding the damage to the self-tissues. Foreign molecules or self-molecules which have the capability of eliciting an immune response are termed as antigens [2]. Immunity may be divided into two parts based on the speed and specificity of the reaction, although they function by interacting with each other, they are the innate immune system and the adaptive immune system [3]. Chaplin’s research [4] states that the innate immune system includes all the defence mechanisms whose mode of action is immediate and acts as a

first line of host defence against the potential pathogen. Earlier in 2003 Chaplin [5] described that the defence mechanism by Innate immune system include the barriers contributed by the epithelial cell layers and the mucociliary blanket that overlays the epithelium in the respiratory, gastrointestinal, and genito-urinary tracts. Parkin and Cohen [3] explains that apart from the barriers, the innate response is majorly encompassed by the immune elements such as neutrophils, monocytes, macrophages, complement proteins, cytokines, chemokines and cell surface receptors that are capable to recognise and bind to the surfaces of the invading microbes [6]. Despite of its speed of action, innate response also causes damage to the normal tissues due to the lack of specificity [3]. Unlike the nonspecific innate response, the mechanism of adaptive immune system is more precise and exquisitely specific to the target molecules involving the T-lymphocytes and B- lymphocytes with antigen specific receptors on their surface, whose presence is vital to distinguish between the self and non-self [4]. T-lymphocytes and B-lymphocytes are further divided into sub populations based on the different functional properties, maturation, and activation [2]. These sub populations of T-lymphocytes and B-lymphocytes are the crucial immune components to evoke an adaptive immune response. Smith and Germolec also [2] also stated that immunocompetent T-lymphocytes usually develop and mature in the thymus and can recognise the antigens only when processed into peptide fragments and are presented as bound to specialised surface molecules called as Major histocompatibility complex (MHC) proteins. T-lymphocytes are involved in cell mediated type of immunity in adaptive response. Whereas, B-lymphocytes are involved in humoral-mediated immunity by producing the antibodies. B-lymphocytes can also recognise the antigens via membrane bound antibody termed as immunoglobulin (Ig) which acts as antigen receptors [2]. The regulation of these interactions between the immune cells and cell products during both the types of immune responses is necessary for the optimal functioning of the immune system [6].

1.2. Self-tolerance and Autoimmune Disease

The mammalian immune system is characterised by having the immense potential for making the receptors that have the capability to sense and neutralize any chemical substance that enters the body [7]. Sometimes, these receptors might recognise the components of our own body by misinterpreting them as foreign. So, the cellular mechanisms have evolved to get rid of these types of aberrant receptors. All these mechanisms contribute to the immunological self-tolerance. self-tolerance can be divided into two types, they are central tolerance and peripheral tolerance [8]. The tolerance mechanisms (in lymphocytes) that are triggered in the central lymphoid organs like thymus and bone marrow before their maturation and circulation are referred to as “central tolerance” [8]. As all the antigens that lymphocytes need to be tolerant of are not expressed in the central lymphoid organs, there are additional tolerance mechanisms that restrains the

lymphocytes that are reactive to the self-antigens that are not expressed in the central lymphoid organs, such tolerance mechanisms that act on the mature circulating lymphocytes in the peripheral lymphoid organs is referred to as “peripheral tolerance [8].” Regardless of tolerance mechanism type, if a lymphocyte (B-cell or T-cell) displays an aberrant self-reactive receptor, then four types of strategies are employed by the immune system to deal with these aberrant receptors [7]. First, the cell that displays self-reactive receptor is subjected to die during the process of clonal selection. Clonal deletion is the process of elimination of the lymphocytes through apoptosis, during which the lymphocytes that show low affinity to the self-antigens are positively selected, whereas the ones that show high affinity are negatively selected in the central lymphoid organs and sometimes in peripheral tissues [8]. Second, in the cell that displays a forbidden receptor, undergoes a mechanism of editing the receptor by certain VDJ recombination or somatic hyper mutation to ensure that a different receptor with no self-reactivity is displayed. This usually happens by the continued expression of RAG1 and RAG2 (Recombination activating genes 1 and 2), which encodes the enzymes for V (D) J recombination to enable the rearrangement of light chain replacement [7]. Thirdly, in addition to the clonal deletion and receptor editing, clonal anergy (functional inactivation) of the forbidden receptors is initiated, which involves two strategies, the intrinsic biochemical tuning of the proteins that bind to the activated BCR (B-cell receptor) for enhancing the B-cell activation and change in the gene expression of the proteins leading to the BCR mediated signalling and activation [7]. If the lymphocytes with forbidden receptors evade all the three mechanisms, collectively contributing to “immunological ignorance”, the fourth strategy of extrinsic regulation is employed, which involves the limiting the supply of growth factors, limiting the co-stimuli, activate suppression by the regulatory T cells and limiting the pro-inflammatory mediators [7, 8]. Thus, these four strategies of immune tolerance act as checkpoints on the pathway leading to the production of antibodies by B cells and effector T cells (see figure 1). Cellular map of various checkpoints regulating the self-reactive receptor bearing lymphocytes is depicted in figure 2. If the cells with forbidden receptors evade these strategies of self-tolerance mechanisms i.e loss of either central or peripheral tolerance, then they are more likely to cause autoimmune diseases [7, 9]. However, there are other mechanisms involved in developing an autoimmune disease. Some of them include the mutation of a regulatory protein called as AIRE, AIRE is an autoimmune regulatory protein that is involved mainly in central tolerance by playing a vital role in the expression of tissue specific self-antigens in thymus [9]. Loss of AIRE function leads to autoimmune polyendocrine disorder. Thus, mutations in various immunoregulatory molecules such as CTLA-4, FOXP3, BIM and death receptors such as FASL and FAS are associated in the development of several autoimmune diseases [9].

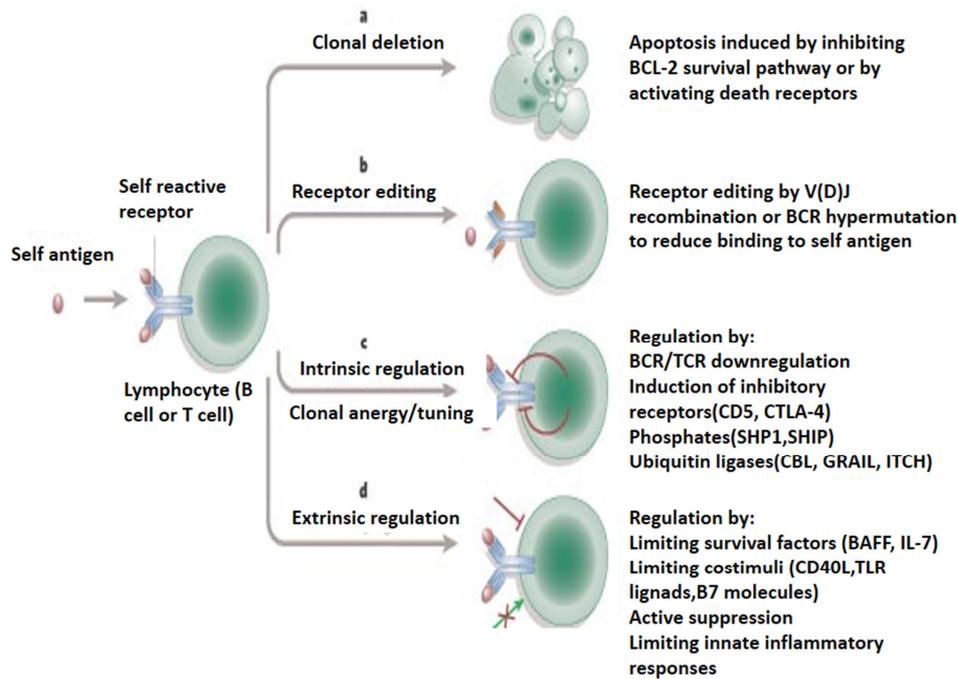


Figure 1. Four cellular strategies to regulate the forbidden or self-reactive receptors. a, Clonal deletion of the cells that display forbidden receptors by inducing apoptosis. b, Receptor editing by V (D) J recombination to enable the receptor to be less self-reactive. c, Intrinsic regulation by biochemical tuning or gene-expression changes to functionally inactivate the receptor's ability to cell activation d, Extrinsic regulation by limiting the growth factors, active suppression by regulatory T cells, limiting costimuli and inflammatory mediators [7].

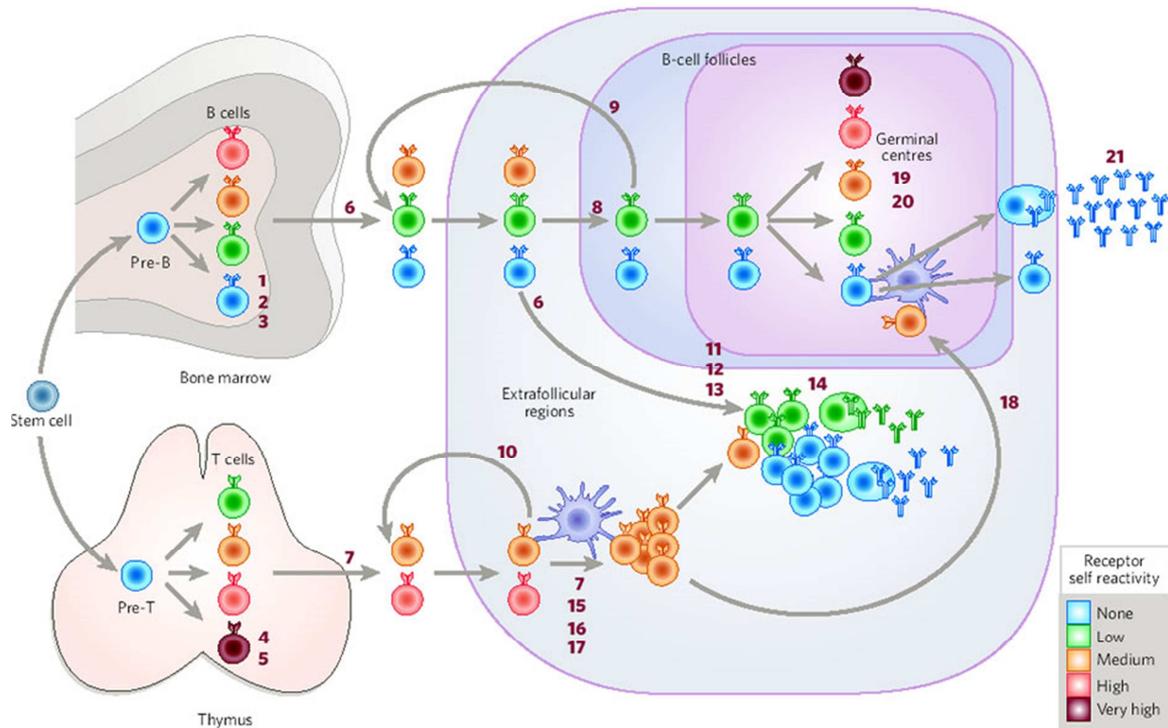


Figure 2. Cellular map of various checkpoints regulating the self-reactive receptors. The numbers in red indicates the specific cellular mechanisms operated by self-tolerance. 1,2 and 3 denotes the various mechanisms operated for B cell receptors in central tolerance: 1) B-cell maturation arrest; 2) B cell receptor editing by V (D) J recombination; 3) clonal deletion. 4 and 5 denotes the various mechanisms operated for T cell receptors in central tolerance: 4) T cell receptor editing by V (D) J recombination; 5) clonal deletion of premature T cells. 6 and 7 denotes the energy of B and T cells, as a part of intrinsic regulation of forbidden receptors. 8 to 17 denotes the mechanisms by extrinsic regulation of self-reactive receptors: 8) exclusion of B cells in follicles; 9) BAFF competition; 10) T cells competition for IL-17; 11) extrafollicular T cells help mediated control; 12) TLR ligands and signalling control; 13) Apoptosis induced in B cells by FASL from T cells; 14) arrest of plasma-cell differentiation; 15) limiting the costimulatory molecules like B7; 16) FASL induced T-cell death; 17) active suppression by regulatory T cells. 18 to 20 denotes the regulation mechanisms by follicular cells. 21 denotes the tolerance mechanisms in final effector phase [7].

1.3. Autoimmune Disease and Its Types

Smith and Germolec [2] conveys that autoimmune diseases usually occur due to the breakdown of immunologic tolerance, that leads to the immune response against the self-tissues. There are two types of autoimmune diseases they are organ specific autoimmune diseases and systemic autoimmune diseases. Organ specific autoimmune diseases are caused by the autoimmune responses that are triggered against the antigens that are confined to a specific tissue [2]. Some of the organ specific autoimmune diseases include Hashimoto thyroiditis, Addison disease, Thyrotoxicosis, Pernicious anaemia and Graves disease. Pohl, D and Benseler, S [9] mentioned that the most common targets for organ-specific immunity are thyroid, stomach, adrenal glands and pancreas. Systemic autoimmune diseases are caused by the immune responses against the self-antigens present in many organs and tissues leading to the massive damage of tissues of the host. Most of the systemic autoimmune diseases affect skin, the joints and the muscle tissue [2]. Some of the systemic autoimmune diseases include scleroderma, rheumatoid arthritis, multiple sclerosis and systemic lupus erythematosus [10]. In certain cases, many autoimmune diseases may occur in a single patient, which are termed as multi-system autoimmune disorders. The different types of autoimmune diseases and their characteristic features are presented in Tables 1 and 2.

2. Systemic Erythematosus Lupus – “A Multisystem Autoimmune Disorder”

Systemic erythematosus lupus (SLE) is a multisystem autoimmune disorder, which is thought to be a result of complex combination of genetic, epigenetic, hormonal and environmental factors [11]. Systemic lupus erythematosus is a multi-system heterogenous disease, in which patient may present it in different ways [12]. Liu and Davidson [13] mentioned that SLE is an autoimmune disease that predominantly affects females and is majorly characterised by inefficient clearance of cell debris and the breakdown of peripheral tolerance mechanisms leading to the production of autoantibodies against the self-antigens and damage to the host tissues and organs [11].

2.1. Aetiology/Pathogenesis of SLE

Despite the advancement in the field of therapeutics and molecular pathology, the aetiology of SLE is poorly understood, which may be attributed to its complex and heterogenous characteristics. However, there are three main defective immune pathways involved in SLE: aberrant clearance of debris containing nucleic acids and/or immune complexes, exaggerated innate immune activation involving TLRs (Toll-like receptors and type-I interferons (IFNs), eventually the abnormal T and B lymphocyte activation [14]. Gaffney, P. M [15] conveys that in SLE, Innate immune is initially

activated by the nucleic acids and this innate response in turn activates the adaptive immune system leading to devastation of the self-tissues mediated by diverse mechanisms. Inefficient clearance of debris results in overload of self-antigens and secondary necrosis, which enhances the access of proinflammatory innate immune receptors of innate immune cells such as activating Fc receptors (FcRs) or TLRs (toll-like receptors) [16]. During the downstream consequences of the TLR signalling, they initiate the transcription of type 1 interferons (IFNs) and other inflammatory cytokines [13, 17]. Gilliet, M [18] describes that TLRs are also involved in activation of MAPK (mitogen activated protein kinase) and other two signalling pathways, that results in abundant production of proinflammatory cytokines such as interleukin-6 (IL-6), IL-12, tumor necrosis factor α (TNF- α) and BAFF (B cell activating factor). These cytokines act as bridge between innate and adaptive immune system by triggering the B cells (auto-reactive) to take up the nucleic acid containing debris and produce antibodies against it. Thus, these immune complexes (nucleic acid containing complexes) are involved in damaging the self-tissues. Due to the loss of tolerance triggered by the aberrant MHC presentation of self-peptides, abnormalities of T cell function are developed during the adaptive immune response in SLE. Thus, aberrant B-cell and T-cell activation contribute significantly in the pathogenesis of SLE.

2.2. Clinical Manifestations of SLE

This disorder was first recognised in 12th century by Rogerius, who applied the term lupus for the classic malar rash and in 1872, Moric Kaposi first recognised the systemic nature of this disease [19]. Systemic lupus is characterised by the presence of varied clinical manifestations. But, the constitutional symptoms include: fatigue, fever, malaise, arthralgias, loss of appetite and weight. The major clinical manifestations of this heterogenous autoimmune disease include: Malar rash, discoid rash, photosensitivity, arthritis (non-erosive), serositis (pleuritis and pericarditis), renal disorder (proteinuria and cellular casts in urine), haematological disorder (haemolytic anaemia, leukopenia, thrombocytopenia), neurological disorder, immunological disorder (with anti-DNA antibodies, anti-Sm antibodies, and anti-phospholipid antibodies) and anti-nuclear antibodies [12]. COJOCARU, M [19] says that according to the diagnostic criteria of SLE, published by American College of Rheumatology (ACR), if a patient said to have any 4 of the above clinical manifestations, then he is more than likely to be suffering from SLE.

2.3. Autoantibodies in SLE

Systemic Lupus Erythematosus (SLE) is a chronic autoimmune and inflammatory syndrome, characterized by a wide spectrum of auto-antibodies against various cellular components [20]. In 1989 Tan, E. M. [21] mentioned that the production of these self-reactive antibodies varies during the course of the disease. Nearly 200 types of autoantibodies were

found in SLE, but the elucidation of the autoantigenic properties, their frequency and correlation with the disease activity were described for 180 autoantibodies [22]. These autoantibodies found in the SLE were produced against the self-antigens such as nuclear antigens, cytoplasmic antigens, cell membrane antigens, phospholipid-associated antigens, blood cells, endothelial cells, and nervous system antigens, plasma proteins, matrix proteins, and miscellaneous antigens [20]. SLE is so far, the autoimmune disease with the largest number of detectable autoantibodies, which may be produced by the result of polyclonal B cell activation (whose self-tolerance mechanism is lost) or due to the aberrant apoptotic pathways [22]. The presence of many autoantibodies specific to self-antigens mainly of nuclear origin (double-stranded DNA (dsDNA), Smith antigen and ribonucleoproteins (Sm/RNP), anti-Sjögren's-syndrome-related antigen A and B (SSA/Ro,

and SSB/La, respectively) is the hallmark of SLE [20, 23, 24]. The major antibodies, their isotypes and correlation with the clinical features are displayed in table 3. Based on the levels of autoantibodies during the development of the disease, three distinct phases are described, they are normal immunity phase, benign autoimmunity phase and pathogenic autoimmunity phase [25]. During the normal immunity phase, patients remain to be asymptomatic by not showing any detectable autoantibody levels [25]. During benign autoimmunity phase, patients are likely to develop significant immunological parameters (ANAs, anti-Ro, anti-La and antiphospholipid antibodies) but lack immediate clinical manifestations [20]. Whereas, the pathogenic autoimmunity phase is characterised by the presence of anti-dsDNA, anti-Sm and anti-RNP and is quickly followed by the onset of SLE specific clinical manifestations. Thus, autoantibody levels can be used to diagnose the severity of the disease [20].

Table 1. Organ specific autoimmune diseases (in humans).

Autoimmune disease	self-antigen	Cause of autoimmunity	Effect of autoimmune response
Autoimmune hemolytic anemia	RBC membrane proteins	Autoantibodies	Haemolysis
Pernicious anemia	a. Intrinsic factor in gastric secretions. b. Gastric parietal cells	Autoantibodies	Interfere with absorption of vitamin B12 (anaemia)
idiopathic thrombocytopenic purpura	platelet membrane proteins	Autoantibodies	platelet destruction
Good pasture's syndrome	Basement membrane antigens of kidney and lung	Autoantibodies	Damage of basement membrane of kidney and lung
Bullous pemphigoid	Basement membrane of skin	Autoantibodies	tense blister formation
Pemphigous vulgaris	Desmoglein-3	Autoantibodies	Blister formation in skin and mucous membrane
Hashimoto's thyroiditis	Thyroid proteins and Thyroid cells	T Cells, Autoantibodies	Destruction of thyroid (hypothyroidism)
insulin-dependent diabetes mellitus (IDDM)	Beta cells of islets of Langerhans in pancreas	T Cells, Autoantibodies	destruction of beta cells (diabetes)
Graves disease	Thyroid stimulating hormone receptors	Autoantibodies	stimulates thyroid (hyperthyroidism)
Myasthenia gravis	Acetylcholine receptors	Autoantibodies	destruction and blocking of acetylcholine receptors

Table 2. Systemic autoimmune diseases (in humans).

autoimmune disease	Self-antigen	Cause of autoimmunity
systemic lupus erythematosus (SLE)	DNA nuclear proteins, cytoplasmic proteins, RBCs and platelet membranes	Autoantibodies, Immune complexes
Rheumatoid arthritis	Joints	Autoantibodies, Immune complexes
Scleroderma	Heart, lungs, kidneys, gastrointestinal tract	Autoantibodies
Sjogren's syndrome	Salivary gland, liver, kidney, thyroid	Autoantibodies
Multiple sclerosis	Brain	Autoantibodies, Tc Cells

Table 3. Major types of autoantibodies, their isotypes and their targets in SLE patients, SSA/B: sjogren syndrome antigen A/B, NMDAR: NMethyl-D-Aspartate Receptor.

Autoantibody type	Isotypes	Pathogenesis involvement	Target antigen
Anti-nuclear antibody	IgG, IgA	Cutaneous lupus	Nuclear membrane, cytoplasmic organelles, cell membranes, and cytoskeletal structures
Anti-dsDNA antibody	IgG, IgG1, IgG2, IgG3, IgG4, IgM, IgE, IgA	Skin and cerebral lupus, lupus nephritis	Nucleosome linker B-DNA, Z-DNA, Phosphodeoxyribose backbone, and kinetoplast DNA
Anti-Nucleosome antibody	IgG, IgG3	lupus nephritis	Histones and DNA
Anti-Sm antibody	IgG, IgG1, IgG3, IgE	Renal, neurologic, vasculitis and haemolytic disorders	core proteins (B, D1, D2, D3, E, F, G)
Anti-RNP antibody	IgG, IgM	–	U1-RNP complex
Anti-Ro/SSA, Anti-La/SSB	IgG3, IgE	Neonatal lupus	Ribonucleoprotein complex with hY (human cytoplasmic) RNAs
Anti-phospholipid antibody	IgG	–	cardiolipin, phosphatidylserine, phosphatidylinositol, sphingomyelin and a phospholipid binding protein cofactor β 2-glycoprotein 1 (β 2GP1)
Anti-C1q antibody	IgG	lupus nephritis	Collagen like region on C1q
Anti-Ribosomal-P antibody	IgG	Liver disease and neuropsychiatric	Ribosomal proteins

Autoantibody type	Isotypes	Pathogenesis involvement	Target antigen
Anti-NMDAR antibody	IgG	symptoms neuropsychiatric symptoms	NMDA receptor

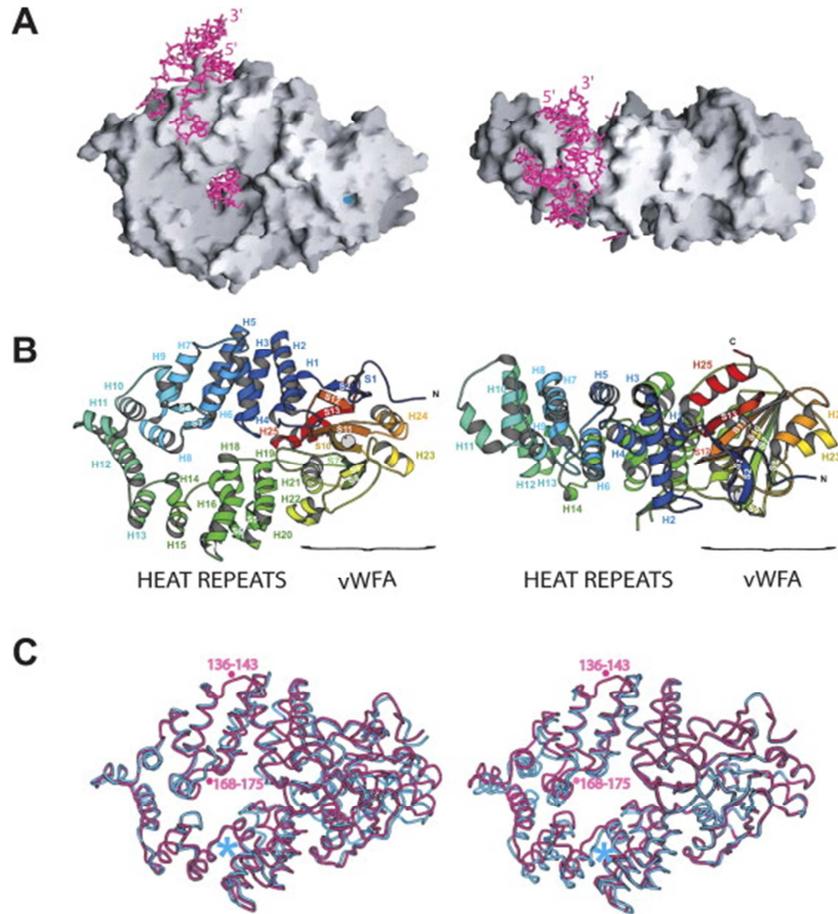


Figure 3. Structures of Ro autoantigen and Ro/RNA complex A) represents the graphical molecular surface of the elliptical toroid like structured Ro60 protein; pink colour indicates the bound RNA. B) corresponds to the horse-shoe shaped domain with HEAT repeats and the vWFA domain, which contains the MIDAS motif coloured in grey spheres. C) superimposed back bone of Ro/RNA complex, 136-143 loop denotes the Y RNA binding surface; 168-175 loop denotes the single stranded RNA binding [27].

3. Ro60 Ribonucleoprotein Complex (Ro-RNP) or Trove-2

3.1. Ro60 Protein Structure and Functions

The Ro 60 kDa Ribonucleoprotein is a ring-shaped RNA-binding protein, that usually binds misfolded non-coding RNAs, pre-5S rRNA, and several small cytoplasmic RNA molecules known as Y RNAs (hY-RNAs) to form a Ro-Ribonucleoprotein complex (Ro-RNP) [26]. Ro60 protein is encoded by a gene called as Trove-2, whose size is about 32 kb and located on the chromosome 1. X-ray crystallography studies have revealed that the Ro60 protein consists of two domains [27-28]. The first domain consisting of series of 7 HEAT repeats (alpha helical repeats) that gives it a horse shoe shape or a doughnut with an inner hole is termed as TROVE domain (see figure 3), which has the capacity to accommodate only single stranded RNAs, but not the double stranded ones [27]. This horse shoe shaped TROVE domain is

clapsed shut by another domain that resembles the von Willebrand Factor A (vWFA) domain of various proteins involved in cell adhesion [27]. This domain consists of a motif known as metal ion-dependant adhesion site (MIDAS), which acts as a cation dependent ligand binding site [27, 28]. Y-RNAs bind to the conserved residues outside the TROVE domain, which were considered to be specific for Y RNAs, but the experiments suggest that these residues also bind the helical portions of the misfolded RNAs that bind inside the central hole of the TROVE-domain [27, 29]. Thus, it suggests the functional role of Y RNAs are thought to block the access of other RNAs to the central cavity. The major functions of this protein include destabilisation of the misfolded helices of RNA that are bound to the central cavity and in recent study, in which human Ro60 was expressed in E. coli had revealed that this protein is involved in correct folding of a misfolded RNAs in vivo [30]. The structure of the Ro60 protein allows it to act as the scaffold for binding helicases or ribonucleases, that are crucial for the RNA quality control pathway for targeting the incorrectly folded noncoding RNAs to decay

[30]. Recent studies of Ro60 protein in mammalian cells suggests that Ro60 is vital for the cell survival after the UV irradiation. Thus, by linking the above functions it is evident that Ro60 is essential for degrading the damaged RNA due to the UV irradiation, because exposure to the UV irradiation might result in RNA: RNA and RNA: Protein crosslinks [27]. The above gain of function is supported by the experiment which resulted in development of lupus like syndrome in the Ro60 knock-down mice by producing antibodies against chromatin and ribosomes [27-29]. Thus, it is evident from the above study that Ro60 is inevitably important for the cells and tissues for preventing the autoimmunity.

3.2. Ro60/Trove-2 and Its Isoforms

Human Trove 2 (Trove domain family member 2) has 6 isoforms (but, sequence and information is elucidated for only 5 isoforms) [28, 31]. Regardless of the sizes of the isoforms, usually the TROVE-2 gene encode for an acetylation site, phosphorylation site, RNA binding region, Trove domain, and von Willebrand Factor A (vWFA) domain with a metal ion dependant acetylation site [30]. The mRNAs for all the isoforms are almost identical at the N-terminus, but they greatly differ at the C-terminus. The canonical isoform (wild type) is a long isoform that has the length of 538 amino acids and encode for a 60 kDa protein, whereas the shorter isoform is of 205 amino acids in length and encodes for a 23 kDa protein. The other three isoforms are at the range of 510 to 530 amino acids in length [27, 28].

3.3. Ro60 and Its Interactions (RNAs and Proteins)

The Ro60 is present in both cytoplasm and nucleus. Ro60 is known to interact with variety of RNA structural elements such as RNA polymerase-3 transcribed Y RNAs (hY RNAs), pre-5S rRNA, RNA motifs derived from the endogenous Alu retroelements [28, 29]. The region where Ro60 binds to Y RNAs is a highly conserved bulged helix and also Ro60 binding to the Y RNA is sequence specific [27]. Regardless of the sequence specificity for binding to the Ro60, misfolded pre-5S rRNAs compete with Y RNA for binding, proving that these two types of RNAs bind to the Ro60 in overlapping way [30]. The interactions of the various proteins with Ro60 in ribonucleoprotein complex is stabilized by Ca²⁺ ions [32]. The protein interactions of the Ro60/TROVE-2 majorly includes with SSB, UBC, TRIM21, TEP1, and PAGR1. SSB (Sjogren syndrome antigen B) or autoantigen La is a protein that usually binds to the 3'- poly U sequence at the terminus of RNA polymerase-3 transcripts to stabilise and protect from them from the nuclease digestion and to promote their proper folding and maturation [28]. TRIM 21 (Tripartite motif containing 21; E3 ubiquitin protein ligase) is the component of ubiquitin ligase complex, which is usually involved in ubiquitination of certain proteins and mediate their degradation [33]. TEP-1 (telomerase associated protein-1) is a component of the telomerase ribonucleoprotein complex that is essential for the telomerase mediated end replication of the chromosomes

[34]. UBC (Ubiquitin C) is a polyubiquitin C precursor involved in ubiquitination of the proteins that may be involved in DNA repair or kinase activity [35]. Thus, the interactions mentioned above suggests that Ro60 is directly or indirectly involved in biological processes like protein degradation, DNA repair and kinase activity.

3.4. Y-RNAs Structure, Types and Their Functional Roles

Inevitably, non-coding RNAs not be encoded into proteins, but play a vital role in many cellular processes. Functions of many types of small non-coding RNAs are yet to be elucidated. Y RNAs are the one of class of small non-coding RNAs, which are conserved in the cells of vertebrates [36]. These small non-coding Y RNAs were first discovered in the Systemic lupus erythematosus and Sjogren syndrome patients, complexed with Ro60 and La autoantigens as a component of ribonucleoproteins (RNPs) [37]. Four types of Y RNAs are discovered in the humans, they are hY1, hY3, hY4 and hY5 RNAs. The genes for all four Y RNAs in humans are present in the form of clusters in a single locus on the chromosome 7q36 [38]. As all the Y RNAs have their distinct promoters, the genes for four Y RNAs are transcribed by RNA polymerase III individually. The sizes of Y RNAs are considerably small with 100 ± 20 nucleotides. Y RNAs are fold into characteristic stem loop secondary structures. The structural analysis had revealed that 5' and 3' ends of RNA are hybridised to form double-stranded upper and lower stem domains with an internal loop (see figure 4) [39]. The nucleotide sequence of the upper and lower stems in all the Y RNAs are highly conserved, whereas the sequence of the internal loop varies between the Y RNAs [36]. The diversely sequenced internal loop has been reported to interact different proteins, such as nucleolin, polypyrimidine tract-binding protein (PTB) and zipcode binding protein 1 (ZBP1), but the functions associated with these interacting proteins is yet to be known [36]. The experiments done by Sim, S [26] suggests that Y RNAs are involved in the sub-cellular localisation of Ro60 and also in its mediated functions by binding to specific types of Y RNAs. X Ray crystallography studies had revealed that Y RNAs bind to the Ro60 with the highly conserved lower stem domain on their outer surface [27]. Y RNAs in collaboration with Ro60 is involved in quality control of RNAs and also in regulating the cellular stress responses (see 3.1).

3.5. Role of Y RNAs in Initiation of Chromosomal DNA Replication

When experimental attempts were made to demonstrate the functions of Y RNAs, then they revealed that Y RNAs have a significant role in the initiation of chromosomal DNA replication. In the experiments conducted by [40], in which cytosolic extracts from the human cells were added to the nuclei prepared from late G1 phase cells had revealed that one particular cytosolic protein was seen to maintain the active replication. This replication factor was found to be non-coding Y RNAs. To validate the gain of function of the Y RNAs,

cytosolic extracts containing the degraded Y RNAs were added to the nuclei, then it was seen that the initiation of the replication was abrogated. Knockout experiments were done to validate the involvement of Ro60 in this function of regulating the initiation of replication, then it was seen that Y RNAs alone were involved in regulating the initiation of replication. Biochemical analysis revealed that Y RNAs were

seen to interact with only replication initiation factors such as CDC6 and CDT-1, but not with the replication fork proteins such as MCM2-7, GINS and polymerases [36]. Thus, it is evident that Y RNAs have a vital role in the replication of the cells. Cancer cells were seen to overexpress Y RNAs [40], suggesting their role in uncontrolled proliferation.

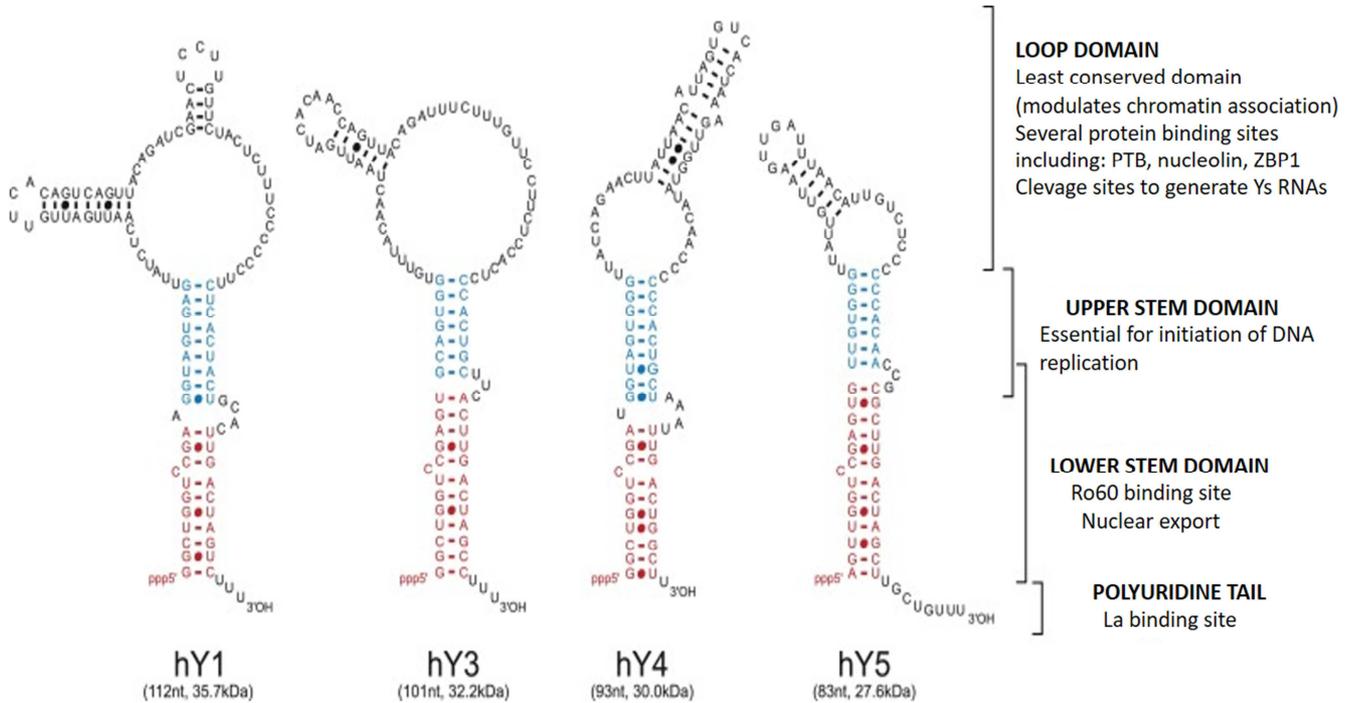


Figure 4. Types of non-coding Y RNAs. All four hY RNAs consists of loop domain, upper stem domain, lower stem domain and a polyuridine tail. Upper and lower stem domains are the conserved regions, having similar nucleotide sequence. All four hY RNAs have different nucleotide sequence at the loop domain. Nucleotide size and weight are represented below each type [36].

3.6. Ro60 Antigenicity and Its Autoantibodies in SLE and Other Autoimmune Diseases

Antibodies against the Ro60 protein were detected majorly in the patients with systemic lupus erythematosus and sjogren syndrome, but they were sometimes reported to be seen in other autoimmune diseases such as systemic sclerosis, dermatomyositis and rheumatoid arthritis [28]. When association between the anti-Ro antibodies and the clinical manifestations of systemic lupus erythematosus and other systemic autoimmune diseases were analysed, then they were likely to have an association with photosensitivity, cutaneous vasculitis and certain haematological disorders, but there is no evidence for the direct involvement of anti-Ro autoantibodies in the pathogenesis of these clinical manifestations [42-43]. Jaccoud’s arthropathy is term used for the relationship established between the anti-Ro antibodies and nonerosive deforming arthritis [27, 28]. Several studies on the epitope recognition by anti-ro autoantibodies had revealed that the epitopes targeted by the anti-Ro autoantibodies in the sera of the SLE patients that predominantly reacting with native Ro60 protein were discontinuous or conformational [27]. Crystallographic studies have shown that the epitope was constituted by two or more parts of a correctly folded protein

[27]. Most of the studies also show that the ant-Ro positive sera are heterogenous in nature, but there are enough studies, that identified an epitope residing between amino acids 169-180 [27-29]. This epitope was reported to be recognised by majority of the patient auto-sera and resides on the loop that bind to the single stranded non-coding RNAs. The major epitopes recognised by anti-Ro antibodies are depicted in the figure 5.

4. Conclusion

Systemic Lupus is one of the autoimmune diseases which usually affects the various tissues and is termed as multisystem autoimmune disease. SLE is a multifactorial disease of unknown aetiology with a variety of presenting features and manifestations. Loss of tolerance to the self-antigens is the vital molecular characteristic of Systemic Lupus Erythematosus, in which pathogenic autoantibodies are produced in the body and causes damage to various organ systems. Despite the advancements in the field of therapeutics, none of the drugs designed by targeting various molecules and cells could not road to the permanent cure but increased the susceptibility to various infections due to the drugs targeting to suppress the immune system. Nearly 200 types of

autoantibodies were detected in the SLE patients, majorly antinuclear antibodies (ANA). The perplexing issue of what allows the body to generate these many types of autoantibodies in an autoimmune disease is an emerging interest to the researchers for better understanding of the disease. Antibodies against a Ribonucleoprotein complex called TROVE-2 (Trove domain family 2) or Ro60 protein were the primitively and prevalently found autoantibodies in the patients with SLE. Trove-2, belonging to the Ro 60-kDa family is a Ribonucleoprotein complex, that usually consists of a horse-shoe shaped TROVE domain, which binds to the small non-coding Y RNAs and is involved in the RNA quality control mechanism. Y RNAs are known to be involved in the initiation of chromosomal replication. Trove-2 has 6 isoforms whose sequence was determined, but recently electrophoretic analysis of the Trove-2 isoforms has revealed that there could be one or two more isoforms of Ro60 may be available, whose sequence is yet to be determined. Thus, experiments should be done to characterise the antigenicity of novel Trove-2 isoforms and their Y-RNA binding capacity in the lupus tissues and other cancer cell lines, such that it might provide useful information for understanding the aetiology mediated by this spliced isoform.

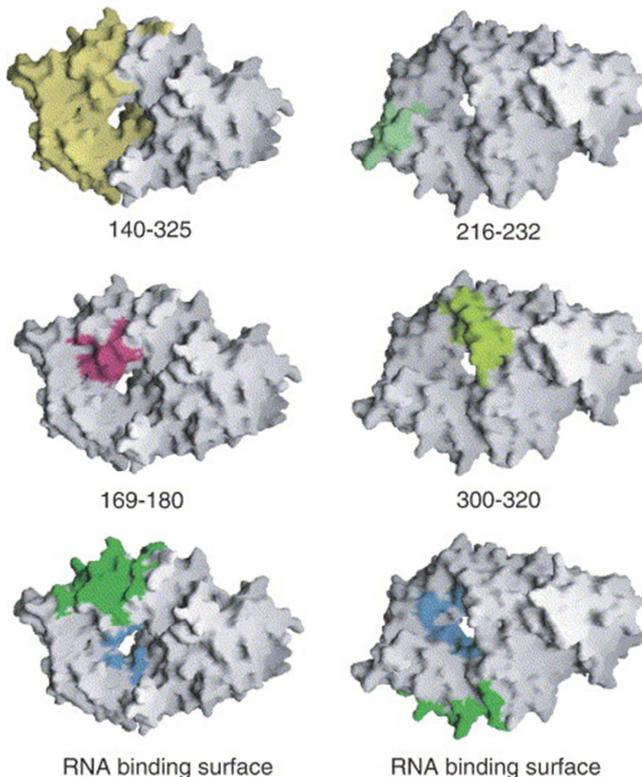


Figure 5. Represents the 3-dimensional structure of Ro autoantigen with epitopes recognised by the autoantibodies from anti-Ro positive sera. Yellow colour indicates the human amino acids 140-325 in the Ro protein, pink indicates the amino acids 169-180, light green denotes amino acids 216-232 and 300-320. The bottom figures show the RNA binding regions. Green indicates the Y RNAs binding region and blue indicates the misfolded RNAs binding region. The anti-Ro autoantibodies binds to the epitope that overlaps with the RNA binding regions [30].

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