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Review

Nucleic acid-associated autoantigens: Pathogenic involvement and therapeutic potential

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abstract

Autoimmunity to ubiquitously expressed macromolecular nucleic acideprotein complexes such as the nucleosome or the spliceosome is a characteristic feature of systemic autoimmune diseases. Disease-specificity and/or association with clinical features of some of these autoimmune responses suggest pathogenic involvement which, however, has been proven in only a few cases so far. Although the mechanisms leading to autoimmunity against nucleic acid-containing complexes are still far from being fully understood, there is increasing experimental evidence that the nucleic acid component may act as a co-stimulator or adjuvans via activation of nucleic acid-binding receptor systems such as Toll-like receptors in antigen-presenting cells. Dysregulated apoptosis and inappropriate stimulation of nucleic acid-sensing receptors may lead to loss of tolerance against the protein components of such complexes, activation of autoreactive T cells and formation of autoantibodies. This has been demonstrated to occur in systemic lupus erythematosus and seems to represent a general mechanism that may be crucial for the development of systemic autoimmune diseases. This review provides a comprehensive overview of the most thoroughly-characterized nucleic acid-associated autoantigens, describing their structure and biological function, as well as the nature and pathogenic importance of the reactivities directed against them. Furthermore, recent advances in immunotherapy such as antigen-specific approaches targeted at nucleic acid-binding antigens are discussed.

1. Introduction

Autoimmune reactivity to antigens that are associated with nucleic acids is a hallmark of systemic autoimmune diseases such as systemic lupus erythematosus (SLE), scleroderma (systemic sclerosis, SSc), mixed-connective tissue disease (MCTD), poly/dermatomyositis (PM/DM) or primary Sjögren's syndrome (pSS). Remarkably, expression of these antigens is not restricted to a single organ or cell type as it is the case in most organ specific autoimmune disorders, but occurs usually throughout the whole body [1,2]. Most autoantibodies (autoAbs) are directed to nucleic acid-binding proteins that are part of large subnuclear complexes such as nucleosomes, centromeres, small nuclear (sn) and nucleolar (sno) ribonucleoproteins (RNPs) or heterogeneous nuclear (hn) RNPs while other autoAbs target particles that are at least temporarily located in the cytoplasm such as Ro RNPs, tRNA synthetase complexes or ribosomes. In the majority of cases the autoAb reactivity is directed to the protein components but sometimes it is the nucleic acid or the complex itself that is targeted. A prototype example of such a complex is the nucleosome where antibodies (Abs) may be directed to individual histones, double-stranded DNA (dsDNA), or DNA-protein complexes [3].

Although a direct pathogenic role has been ascribed to only a few of these autoAb-families, some of them show a striking disease-specificity or are associated with certain clinical features which may be considered indirect evidence for pathogenic involvement [1,2]. For example, antibodies to nucleosomes or dsDNA are very specific for SLE and correlate with disease activity and clinical features [4], antibodies to topoisomerase-I are highly specific for diffuse scleroderma and associated with an unfavourable prognosis, while antibodies to centromere proteins are preferentially found in the sera of patients with limited scleroderma which is a relatively mild disease [5]. Other autoAbs are not strictly disease-specific such as anti-Ro antibodies which are frequently present in the sera of SLE patients as well as in the majority of sera from patients with pSS but also in up to 10% of patients with other systemic diseases. However, the lack of disease-specificity does not necessarily exclude a pathogenic role. For example, antiphospholipid Abs are not specific for the antiphospholipid syndrome but are nevertheless among the pathogenic key players of this condition. Furthermore, neonatal lupus erythematosus is caused by the transplacental passage of maternal autoAbs, in particular anti-Ro52 autoAbs, that cross-react with the

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heart 5-HT_4 serotoninergic receptor [6]. Moreover, even if autoAbs represent only epiphenomena, the underlying T cell responses may be essential in the pathogenesis of systemic autoimmune disorders by virtue of production of proinflammatory cytokines and/or by exerting cytotoxic functions. Classical T cell reactivity is directed to proteins and autoreactive T cells recognizing peptides derived from proteins, of the complex drive also the humoral immune response to nucleic acids. Thus, histone-specific T cells are known to drive the autoAb response to dsDNA in SLE, which is both disease-specific and of pathogenic relevance, while histone-reactive T cells may also be present in healthy subjects [7].

The reason for selection of nucleic acid-binding and ubiquitously expressed antigens as autoantigen targets has remained an enigma for many years. With the discovery of pattern recognition receptors able to bind nucleic acids and crucially contributing to disease pathogenesis upon activation, it has become evident that the selection of these particles for immune attack is based on their intrinsic ability to activate those nucleic acid-binding receptor systems. Involvement of Toll-like receptors (TLRs) in lupus-like autoimmune conditions has clearly been demonstrated and there is increasing evidence that TLRs and other nucleic acid sensors may play crucial roles also in the pathogenesis of various connective tissue diseases.

In the following sections we will describe structure and biological function of the most important and most thoroughly-characterized nucleic acid-associated autoantigens, and discuss their role as diagnostic markers and drivers of potentially pathogenic autoimmune responses. Furthermore, recent advances in immunotherapy including antigen-specific approaches and targeting of intracellular molecules that interact with nucleic acids, such as TLRs, will be presented. A better understanding of the disease processes will make possible the development of novel therapeutic concepts that may allow treating the diseases more effectively in their earliest stages, to induce remission, and to achieve definite cure.

2. DNA-associated autoantigens

2.1. The nucleosome

Nucleosomes are the basic repeating units of eukaryotic chromatin, i.e. the highly organized and complex form in which genomic DNA is packed into the nucleus. Histones, the major protein components of chromatin, act as spools around which DNA winds and thus have a role in gene regulation by condensing and decondensing DNA. Apart from autoAbs to double-stranded DNA and to the individual histone proteins, autoAbs to native nucleosome structures can be found in the sera of patients with SLE and, at lower levels, of other diseases. In SLE, these autoAbs form immune complexes with nucleosomes and are assumed to be primarily involved in the development of lupus nephritis.

2.1.1. Size of autoantigens

Histone H1 (main isoform H1b): 218 aa (21.4 kD); Histone H2A: 129 aa (14.0 kD); H2B: 125 aa (13.7 kD); H3: 135 aa (15.3 kD); H4: 102 aa (11.2 kD).

2.1.2. Structure

Each core particle consists of a central histone octamer unit containing 2 copies each of the core histones H2A, H2B, H3, and H4 around which 146 base pairs of genomic dsDNA is wound. The length of the linker DNA between two core particles is 8e114 base pairs. The extranucleosomal linker histone H1 resides at the DNA entry site and locks the DNA into place. The 4 'core' histones (H2A, H2B, H3 and H4) are relatively similar in structure and are highly conserved in evolution, all featuring a 'helix turn helix turn helix' motif (which allows the easy dimerization). Histone tails are mobile and highly basic which is essential for their interaction with DNA.

2.1.3. Related proteins

Histone proteins are among the most highly conserved proteins in eukaryotes, emphasizing their pivotal role in the biology of the nucleus. In addition to the major histone forms which build up the nucleosome there exist numerous variants (reviewed in [8]) that share primary sequence homologies and core structural similarities with the major histones but also have their own characteristic features that distinguish them from major histones. These minor histones usually carry out specific functions in chromatin metabolism. For example, histone H3-like CENP-A is exclusively associated with the centromere region of the chromosome. Histone H2A variant H2A.Z is associated with the promoters of actively transcribed genes and also involved in heterochromatin formation. Another H2A variant, H2A.X, binds to DNA with double-strand breaks and thereby marks regions undergoing DNA repair. Histone H3.3 is associated with the body of actively transcribed genes. Certain variants such as $H1^o$, H5, H1t, TH2B, H2BFWT or H3t occur in specific tissues only.

2.1.4. Expression

Histones are found in the nuclei of all eukaryotic cells, and in certain Archaea, but not in bacteria. Interestingly, yeast cells do not contain the linker histone H1.

2.1.5. Location

Nuclear.

2.1.6. Biological function

Nucleosomes have two major functions: 1. Compaction of DNA (7-fold compaction in nucleosomes, up to 30,000-fold compaction in higher-ordered chromatin structures) that enables to fit the large genomes of eukaryotes inside cell nuclei. 2. Transcriptional regulation. Transcriptionally active euchromatin has a less compact structure compared to transcriptionally inactive heterochromatin. Compaction and activity are modulated by replacing core histones with other histone variants or by the numerous reversible post-translational modifications of specific amino acid residues, occurring essentially within the N-terminal histone tails that protrude outside from the nucleosome core particle.

2.1.7. Knockout

Core histones are absolutely essential for cell viability. The linker histones are dispensable in lower unicellular eukaryotes but have been shown to be essential in mice (reviewed in [9]). Furthermore, during the late haploid phase of male germ cell maturation, most histones are replaced by protamines responsible for the tight compaction of the sperm DNA [10].

2.1.8. Modifications

Histones are heavily post-translationally modified primarily at their N-terminal tails, but also in their globular domains. Modifications include methylation, deimination (citrullination), acetylation, phosphorylation, sumoylation (covalent attachment of SUMO protein), ubiquitination, and ADP ribosylation. Histone-modifications are essential in diverse biological processes such as gene regulation, DNA repair and chromosome condensation during mitosis. Combinations of modifications are considered to constitute the so-called histone code [11,12].

2.1.9. Autoantibodies

Abs can be directed to dsDNA, single histones, histone complexes such as the H2A-H2B dimer, and conformational epitopes of the entire nucleosomal protein-DNA complex. The latter are named

anti-nucleosomal Abs and have no or very low reactivity against individual histones or non protein-complexed dsDNA. In particular, H2A-H2B dimers are frequently recognized by anti-histone Abs (AHA), especially in drug-induced LE [11,13,14]. AHA can be found in a variety of diseases, including inflammatory hepatic [15], malignant, infectious [16] and, of course, rheumatic autoimmune diseases [17] (Table 1). They may be present in up to 75% of SLE sera and in more than 90% of drug-induced lupus, while their prevalence in other rheumatic diseases usually does not exceed 20% [18,19]. High titers of AHA appear to be relatively specific for SLE [20]. Sequential as well as backbone determinants of DNA can be targets of anti-DNA Abs. In contrast to most other antigen–antibody interactions, the binding of Abs to DNA is predominantly based on electrostatic interactions. Anti-DNA Abs react with DNA of all species, probably because the sugar-phosphate backbone is the major target structure. However, selective recognition of different DNA molecules might be based on recognition of specific nucleotide sequence motifs. A subpopulation of anti-nucleosome autoAbs recognizes highly structural epitopes (quaternary epitopes) composed of histone as well as DNA elements [21,22].

2.1.9.1. Role in diseases. Increased apoptosis or insufficient phagocytosis of dying cells by macrophages in lupus patients may lead to the release of nucleosomes into the extracellular space where they may be engulfed by dendritic cells (DC) that can present them in an immunogenic manner to T cells, which subsequently stimulate B cells to produce autoAbs and initiate immune complex (IC) formation. ICs then are targeted to basement membranes, especially the glomerular basement membrane where positively charged histones bind to negatively charged heparan sulfate residues of the basement membrane [23]. This triggers the development of local inflammation, resulting in lupus nephritis and kidney damage. Similar to anti-DNA Abs, anti-nucleosomal Abs and AHA correlate with lupus disease activity [24–26], particularly Abs to histone H1, which appear to be more specific for SLE than other AHA [18,20]. Of note, it is anti-H1 Abs that are responsible for the LE cell phenomenon, which was originally included into the American College of Rheumatology (ACR) classification criteria for SLE [27,28].

High avidity IgG anti-dsDNA Abs are associated with lupus nephritis while an IgG/IgM ratio below 0.8 has been reported to be associated with the absence of renal involvement [29]. Furthermore, anti-dsDNA Abs may pass the placenta and are associated with transient neonatal lupus syndrome. Interestingly, anti-TNF therapy can induce anti-dsDNA Abs in RA patients that are mostly of the benign IgM isotype.

2.1.10. Autoreactive T cells

AutoAbs to chromatin components show Ig class switching and affinity maturation which are typical features of a T cell-dependent Ag-driven immune response [30–32]. Histones are considered as the major T cell antigens in SLE for the generation of autoimmune responses against chromatin components: histone-specific T-cell clones augmented the production of anti-dsDNA, anti-ssDNA and AHA in murine models of lupus [33] and also promoted anti-dsDNA production by autologous B cells in human SLE [34,35]. Histone H1 may be the most important single histone autoAg, both at the B and T cell level. All histones elicited a Th1-like response in human peripheral blood mononuclear cells (PBMC), inducing high production of IFNγ and TNFα, but no IL-4, and only little IL-10 [20].

By using overlapping 15-mers or by eluting peptides from MHC molecules, the critical T cell autoepitopes of core histones and histone H1 were identified. The recurrent T cell autoepitopes in human SLE overlap with the major autoepitopes for nephritogenic T cells in lupus-prone SNF_1 cells [7,36,37] (Fig. 1). Interestingly, both in mice and humans these epitopes are located in the histone regions that are also targeted by lupus B cells and sites that contact with DNA in the native nucleosome particle [38].

2.1.11. Therapy

Immunosuppressive therapy suppresses the development of anti-nucleosomal and anti-dsDNA Abs in patients with SLE. Plasmapheresis initially dramatically reduces anti-dsDNA Ab levels, but no differences are observed in the long-term final outcome. The major peptide autoepitopes can be promiscuously presented and recognized by T cells from SLE patients in the context of diverse MHC alleles. This advantageous feature opens up the possibility for developing universally tolerogenic peptides for therapy in human SLE. Thus, administration of single 15–20 residue-long histone peptides were found to halt the progression of established glomerulonephritis in SNF_1 and (NZB × NZW)F1 mice [39,40].

In another approach, a peptide derived from the complementarity-determining region (CDR)-1 of an anti-DNA Ab ameliorated disease in several lupus mouse models [41]. In a clinical pilot study involving 9 SLE-patients treated for 26 weeks with this peptide, downregulation of inflammatory cytokines and of pro-apoptotic molecules was observed, and disease activity was reduced significantly [42].

Table 1
AutoAbs to components of the nucleosome.

Abs to any nucleosomal component	
Systemic lupus erythematosus	~75%
Other autoimmune diseases	<5%–50% (AHA)
Abs to individual components of the nucleosome in SLE	
Nucleosome-restricted Abs (structural epitopes)	31–100%
Anti-dsDNA Abs	21–82%
Anti-histone Abs	30–75%

2.2. Centromere-associated autoantigens

The centromere is a chromosomal region involved in cell division and the control of gene expression. It usually contains characteristic types of DNA sequences, in higher eukaryotes typically tandem repetitive sequences, often called “satellite DNA”. These sequences bind centromere-specific proteins (CENPs). During mitotic division, a transient structure called kinetochore is formed on top of the centromeres that mediates the movement of the two sister chromatids to opposite poles of the dividing cell nucleus along the fibers of the spindle apparatus. Anti-centromere antibodies (ACA) are associated with a limited form of scleroderma and a more favorable disease course.

2.2.1. Size of major centromere autoantigens

CENP-A: 140 aa (17 kD); CENP-B: 594 aa (80 kD); CENP-C: 943 aa (140 kD; anomalous migration at 140 kD).

2.2.2. Structure

CENP-A contains a 93 aa C-terminal domain that shares 62% aa-homology with histone H3 and is responsible for its chromatin location, and a divergent NH_2-terminal domain.

CENP-B contains two DNA-binding boxes in the N-terminus that localize the protein to the centromere and a dimerization domain at the C-terminus. In the central part of the protein there is a non-functional DDE-endo-nuclease domain. CENP-B builds homodimers that are thought to bind to two distant CENP-B boxes during centromeric heterochromatin formation.

CENP-C includes a central DNA-binding domain (centromere-targeting) without any homology to known DNA-binding domains. The N-terminus contains a unique domain that is responsible for

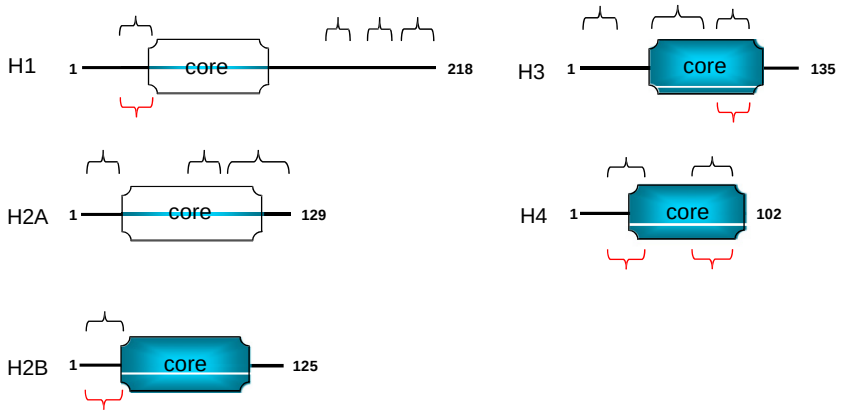

Fig. 1. Domain organization and epitopes of the histone autoantigens. The recurrent autoepitopes identified for T cells of SLE patients (red parentheses), namely H2B aa 10e33, H3 aa 95e105, H4 aa 16e39, H4 aa71-94, overlap with the major epitopes in lupus-prone SNF1 mice and lie in regions that are also targeted by SLE patients' autoAbs (black parentheses). The same sequences are also subjected to post-translational modifications. The highly complex code of histone-modifications is not shown in the graph. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

destabilization of CENP-C not assembled at the kinetochore, thus preventing toxic accumulation of CENP-C. The C-terminal portion of the protein is highly homologous to Mif2, a protein of Saccharomyces cerevisiae required for the correct segregation of chromosomes, and also encompasses a Cupin domain (a b-barrel-shaped domain of unknown function) (Fig. 2).

2.2.3. Related proteins

CENP-D, CENP-E, CENP-F, CENP-G, CENP-H. The family of CENPs is highly conserved within eukaryotes.

2.2.4. Expression

CENPs are abundant ubiquitous proteins whose expression changes markedly during the cell cycle according to their function. CENP-A and CENP-B expression peaks in the G2 phase [43,44] while CENP-C expression is increasing from the S phase through G2 phase and mitosis, showing the highest abundance in the G1 phase [45]. In immunofluorescence, anti-CENP-ABC antibodies produce a characteristic speckled pattern, with the speckles distributed uniformly throughout the entire interphase nucleus, recognizing paired sister chromatids assembled at the metaphase plate. In mitotic cells these speckles are found in the condensed chromosomal material [46].

2.2.5. Location

Nucleus (bound to centromeric DNA). CENP-C is additionally accumulated at the nucleolus [47].

2.2.6. Biological function

CENP-A is a component of a modified nucleosome or nucleosome-like structure in which it replaces histone H3 in the core of the nucleosome particle [48], thus providing a high structural rigidity to the centromeric nucleosomes that is likely to be essential for maintaining centromere structure and function [49].

CENP-B is located within the centromere heterochromatin and binds to a 17-bp motif of the CENP-B box sequence that is found in centromere-specific DNA. Recently, CENP-B released in the extracellular medium was shown to exhibit cytokine-like effects on smooth muscle cells, inducing secretion of IL-6 and IL-8 [50].

CENP-C colocalizes with CENP-A at the inner plate of the kinetochore and is necessary for normal kinetochore assembly [51] and chromosome segregation [52], but may also have an additional role related to cell cycle control [45].

2.2.7. Knockout

A CENP-A knockout is embryonally lethal due to severe mitotic dysfunction [53]. CENP-B^{ko}-mice appear normal but have reduced

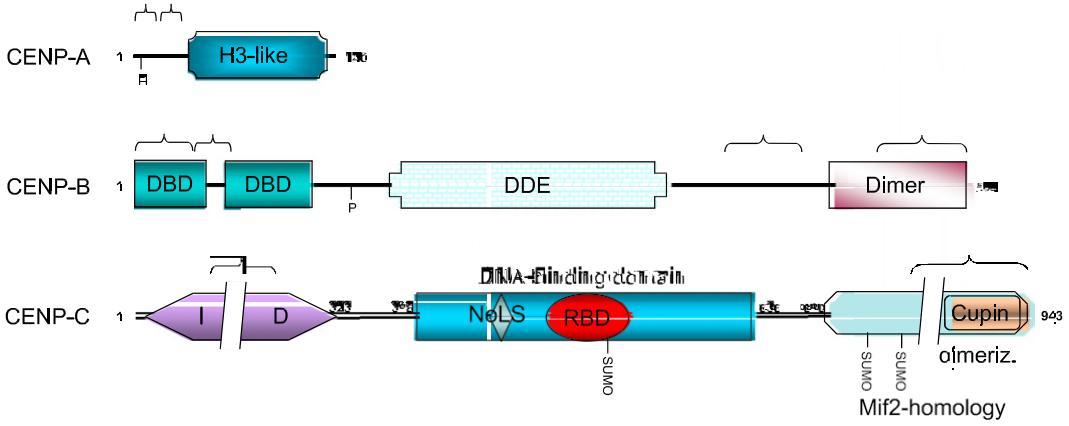

Fig. 2. Domain organization and epitopes of the major centromere autoantigens CENP-A, CENP-B, and CENP-C. CENP-A contains several linear B-cell epitopes within two antigenic N-terminal regions (aa 2e17 and 22e38). The conformational major epitope of CENP-B is located within the extreme C-terminal region (recognized by all ACA-containing sera). Major B-cell epitopes on CENP-C are present within aa 72e244 and 820e943. Cupin, barrel-shaped Cupin domain; DBD, DNA-binding domain; DDE, non-functional endo-nuclease domain; Dimer, dimerization domain; H3-like, histone H3-like domain; ID, Instability domain; NoLS, nucleolar localization signal; P, phosphorylation site; RBD, RNA-binding domain; SUMO, sumoylation site. B-cell epitopes are shown in black parentheses.

body weight and reduced gonad weight and exhibit limited fertility [54]. CENP-C^{ko}-mice show an embryonic lethal phenotype due to mis-segregation of chromosomes [55].

2.2.8. Modifications

The histone-variant CENP-A exhibits a spectrum of histone-modifications that is distinct from both euchromatin and flanking heterochromatin [56]. CENP-C is sumoylated on three lysine residues located in the DNA-binding and the Mif2-homology domain [57] (Fig. 2).

2.2.9. Associated nucleic acids

CENP-A and CENP-B are associated with chromosomal centromeric DNA. CENP-B binding is relatively specific for a 17-bp sequence within a-satellite DNA that is called the CENP-B box and contains two CpG dinucleotides [58]. CENP-C is a dual DNA/RNA-binding protein that additional binds a-satellite RNA [47].

2.2.10. Autoantibodies

Anti-CENP-A, CENP-B and CENP-C autoAbs are partially cross-reacting and are frequently found together in autoimmune sera with CENP-B being the immunodominant antigen. ACA are found in 70e80% of patients with a limited cutaneous form of SSc formerly known as CREST syndrome while their incidence is only 20e35% in diffuse SSc. They may also occur in approximately 5% of SLE patients and in up to 10% of patients with pSS but are very rare in other autoimmune diseases. Interestingly, CENP-C may be a more specific target in pSS (Table 2). ACA are also found in about 80% of sera of tight skin-2 mice which are a model of SSc [59]. AutoAbs to other CENPs are less common. Interestingly, autoantibodies to CENP-F appear to be associated with certain forms of cancer (non-Hodgkins lymphoma, breast and lung cancer) [60,61].

2.2.10.1. Epitopes. B-cell epitopes on CENP-A are restricted to the N-terminal region [62,63]. AutoAbs to CENP-A target two immunologically related linear epitope motifs which cross-react with related motifs on other nuclear autoantigens and on the Epstein e Barr nuclear antigen I [64]. CENP-B epitopes are located at both the DNA-binding N-terminus and, more importantly, the C-terminus [65]. Major CENP-C epitopes lie within the instability domain at the N-terminus and the dimerization domain at the C-terminus [66] (Fig. 2).

2.2.10.2. Role in diseases. Neither a direct pathogenic role nor a correlation with disease activity of ACA is known. Instead, they appear to be associated with a more favorable disease outcome. In contrast to sera containing antibodies to topoisomerase-I, ACA-positive sera only rarely induce IFNa-production [67]. However, ACA are often connected with the development of connective tissue disease, for example in Raynaud's phenomenon [68]. The presence of ACA might also be a risk factor for pulmonary hypertension [69]. Recently, CENP-B released in the extracellular medium was shown to exhibit cytokine-like effects on smooth muscle cells, inducing secretion of IL-6 and IL-8 [50].

2.2.11. Autoreactive T cells

Not known.

2.2.12. Therapy

Due to the unknown role of CENPs in disease etiology, no therapeutic studies have been performed.

2.3. DNA Topoisomerase-I

Autoantibodies to Topoisomerase-1 (Topo-I) are specific serological markers for systemic sclerosis, particularly for diffuse scleroderma, and are associated with disease severity and an unfavourable prognosis. They are often directed to a 70-kD degradation product containing the catalytic domain of the Topo-I protein and were therefore initially termed anti-Scl-70 antibodies.

Table 2
Anti-centromere autoantibodies.

Scleroderma	20e35%
Limited Scleroderma (CREST)	64e95%
Diffuse Scleroderma	0e14%
Sjögren's syndrome	10%
Systemic lupus erythematosus	5%
Non-Hodgkins lymphoma	7%

2.3.1. Size of the autoantigen

The size of the whole Topo-I antigen is 765 aa residues (105 kD). Smaller proteolytic products ranging from 60 to 100 kD can also be enzymatically active. The mass of the catalytic C-terminal domain that was initially identified as Scl70 autoantigen in humans is 67.7 kD.

2.3.2. Structure

Four major domains can be distinguished: the NH_2-terminal domain (aa 1e200), a global core (200e635), a linker domain (636e712), and the COOH-terminal domain (713e765). The global core and the C-terminal domain are responsible for the catalytic activity of Topo-I. The enzymatically active site of human Topo-I contains a critical tyrosine residue at position 723 [70].

2.3.3. Evolutionary conservation

Topoisomerase-I shows a significant degree of conservation among different species.

2.3.4. Expression

Although present in only a single copy in the human genome, Topo-I is a housekeeping protein present in relatively high amounts in the nucleus of all eukaryotic cells.

2.3.5. Location

Nucleus.

2.3.6. Biological function

Topo-I catalyzes the conversion of DNA topologic forms by creation of transient ssDNA nicks and religation, and relaxes supercoiled DNA during essential cellular processes such as replication, recombination, transcription, and DNA repair. In a reaction intermediate the 3'terminus of the nicked DNA strand is covalently linked by a phosphodiester bond to the OH-group of tyrosine residue 723 of the enzyme [71].

2.3.7. Knockout

Yeast mutants containing gene disruptions in Topo-I have no obvious defects, demonstrating that Topo-I is not essential for viability in yeast [72]. In yeast, topoisomerases I and II can at least partially substitute for each other in transcription and replication. However, cells treated with the alkaloid camptothecin, a specific inhibitor of eukaryotic Topo-I, exhibit accumulation of positive supercoils in DNA that goes along with a reduced rate of transcription by both RNA polymerase I and II [73].

2.3.8. Modifications

Topo-I appears to be highly susceptible to proteolytic cleavage by cathepsins during cell death, especially necrosis. Topo-I is cleaved into 70 kD and 45 kD-fragments in endothelial cells undergoing necrosis. Thus, dying endothelial cells could serve as reservoirs of

potentially immunogenic fragments of Topo-I in SSc [74]. Phosphorylation at certain serine residues is required for the activity of the enzyme [75] (Fig. 3).

2.3.9. Autoantibodies

Anti-centromere autoAbs (ACA) are found in specific serological markers for SSc, especially for the diffuse cutaneous form of the disease. They occur in 20e30% of SSc patients, in 20e60% of the diffuse cutaneous scleroderma subset and 46e56% of those with lung involvement (Table 3). The presence of ATA is associated with HLA-DR5 and HLA-DRB1*11 [5]. Apart from SSc, ATA are occasionally found in SLE patients [76]. Interestingly, anti-centromere and ATA appear to be mutually exclusive, occurring together in only 5% of sera [5]. ATA have been shown to inhibit the activity of Topo-I in vitro.

2.3.9.1. Epitopes. Epitope mapping studies based on Topo-I recombinant fragments as well as overlapping 20-mer peptides identified several epitopes in the central portion of the protein [77e80] that is included in the C-terminal 70 kD-fragment (Fig. 3). Thus, this fragment is apparently processed during cell death which may form a molecular basis for initiation of an immune response [74].

2.3.9.2. Role in diseases. ATA can be found in sera several weeks before the development of overt clinical SSc arguing for a direct correlation with pathogenic events. The presence of ATA in patients with SSc comes along with an increased mortality rate and is associated with global disease activity and a poor clinical course. More precisely, there are associations with the severity of cutaneous involvement, pulmonary interstitial fibrosis and cardiac involvement [80,81]. Interestingly, ATA may induce IFNa-production in plasmacytoid DC from SSc patients. The higher IFNa-levels measured in the sera from patients with diffuse SSc as compared to those with limited SSC as well as in the sera from patients with lung fibrosis suggests that IFNa may directly contribute to tissue injury [67]. The pathogenic process in SSc might be associated with the expression of cryptic determinants on Topo-I that might be created by overexpression and/or structural modifications occurring particularly in affected patients.

2.3.10. Autoreactive T cells

Several T-cell epitopes of the Topo-I have been identified and evidence has been found to suggest epitope spreading to occur in patients with SSc [82] (Fig. 3). The autoantigenic response is dependent on both the type of the processed antigen (e.g. full-length or truncated Topo-I) and the type of the antigen-presenting cell. In vivo, cryptic epitopes from fragmented forms of Topo-I seem to be mainly responsible for initiating autoreactive T cell responses [83]. However, autoreactive T cells showing different peptide specificities were also found in the blood of healthy donors with matching HLA-DR alleles [82,84].

Table 3
Autoantibodies to DNA topoisomerase-1 in systemic sclerosis.

All SSc	20e30%
Diffuse scleroderma	20e60%
Limited cutaneous SSc	<10%
Diffuse cutaneous SSc	39e80%
SSc with lung involvement	46e80%

2.3.11. Therapy

No therapeutic approaches interfering with autoimmunity to Topo-I have been published yet.

2.4. Ku/DNA-PKc

The Ku protein was recognized more than 25 years ago as targets of autoantibodies in a subset of Japanese patients with scleroderma-polymyositis overlap syndrome, and anti-Ku antibodies have since been shown to occur in 10e20% of patients with this and other systemic rheumatic diseases, including systemic lupus erythematosus. Ku functions physiologically in the repair of DNA double-strand breaks, where it carries out the initial recognition of damaged DNA ends.

2.4.1. Size of autoantigens

Ku70: 609 aa (69.7 kD); Ku80: 732 aa (82.6 kD); DNA-PKcs: 4096 aa (360 kD).

2.4.2. Structure

The Ku autoantigen is a heterodimer of Ku70 and Ku80 that builds a basket-like structure binding to the termini of dsDNA [85]. The C-terminal portions of Ku70 and Ku80 are responsible for DNA-binding whereas the N-terminal portions are thought to be involved in interactions with other proteins putatively mediated via a von Willebrand factor A domain. Upon DNA-binding the catalytic subunit DNA-PKc is recruited to the complex, thus building a holoenzyme referred to as DNA-dependent protein kinase.

2.4.3. Related proteins

No significant sequence homology with other proteins.

2.4.4. Expression

Ku homologues are present in insects and yeast as well as in bacteria [85]. In primates Ku is detectable in nearly all cell types with the exception of mature neutrophils.

2.4.5. Location

Nucleus and nucleoli of most cells.

2.4.6. Biological function

Ku/DNA-PKc is responsible for DNA non-homologous end-joining (NHEJ), thus for repairing dsDNA breaks and for maintenance

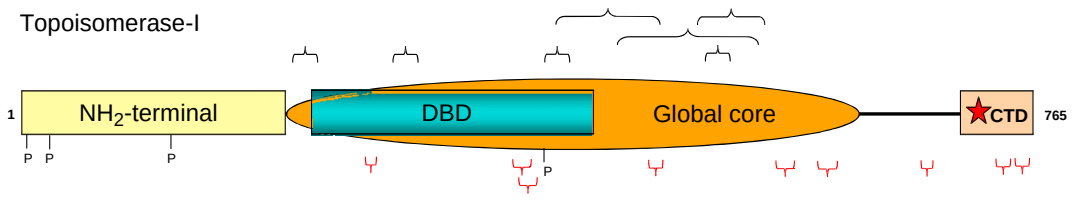

Fig. 3. Domain organization and epitopes of the scleroderma autoantigen DNA Topoisomerase-I. The region encompassing major conformational B-cell epitopes (recognized by the majority of Topo-I positive sera) spans aa 489e700. Additional linear epitopes are spread over the central portion of the protein. T cell autoantigenic regions have been identified in the central and C-terminal parts of Topo-I. CTD, C-terminal domain; DBD, DNA-binding domain; P, phosphorylation site. Major B-cell epitopes are depicted in black parentheses, T-cell epitopes in red parentheses. The enzymatically active site is indicated by a red asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of telomeres. In addition to binding to exposed DNA termini and activation of DNA-PK, the Ku heterodimer plays a role in mammalian NHEJ that is regulated by binding of inositol hexakisphosphate [86].

2.4.7. Knockout

Ku70-deficient animals are sensitive to ionizing radiation and deficient in V(D)J rearrangement, leading to severe immunodeficiency [87]. The absence of Ku also leads to shortening of telomeres and increased rates of chromosome end fusion [85].

2.4.8. Modifications

Ku70 and Ku80 are both phosphorylated in vivo by the recruited DNA-dependent protein kinase [88] (Fig. 4).

2.4.9. Autoantibodies

AutoAbs to Ku/DNA-PKC are mostly found in scleroderma-myositis overlap syndrome and SLE [89,90]. The clinical associations as well as the prevalence differ by ethnicity (Table 4). AutoAbs specific for Ku70, Ku80, or for epitopes created by dimerization have been reported. The latter appear to stabilize the heterodimer. In contrast, binding of autoAbs to DNA-PKc results in its dissociation from the Ku dimer.

2.4.9.1. Epitopes. Major B-cell epitopes of Ku70 and Ku80 are localized within their extreme C-terminal region [91] (Fig. 4). In the case of Ku70, the major epitope colocalizes with the DNA-binding domain. Minor conformational epitopes are spread over the whole molecule.

2.4.9.2. Pathogenic relevance. There is limited evidence of direct pathogenic relevance or association to disease activity.

2.4.10. Autoreactive T cells

None detected yet.

2.4.11. Therapy

None established.

Table 4
Anti-Ku autoantibodies.

Scleroderma	4% (AfricaneAmerican) 3% (Japanese) 0% (Caucasian)
Polymyositis/dermatomyositis	4% (AfricaneAmerican) 4% (Japanese) 0% (Caucasian)
Connective tissue disease overlap syndromes	30% (Japanese) 1% (Caucasian) 0% (AfricaneAmerican)
Systemic lupus erythematosus	9% (AfricaneAmerican) 6% (Japanese) 0.6% (Caucasian)

2.5. Poly(ADP-ribose) polymerase

Poly(ADP-ribose) polymerase (PARP) is a zinc-finger DNA-binding enzyme, which detects and signals DNA strand breaks or nicks. In the cell, it plays a pivotal role, particularly in the maintenance of genomic DNA stability, apoptosis and in the response to oxidative stress. Since these situations are found in cancer, inflammation, autoimmunity, myocardial dysfunction, certain infections, ageing and radiation/chemical exposure, attempts have been made to modulate PARP activity using both inhibitors and activators according to the physiopathological situation. Naturally-occurring antibodies to PARP and its product poly(ADP-ribose) have been detected in various autoimmune diseases, and notably in SLE.

2.5.1. Size of autoantigen

PARP-1: 1014 aa (113 kD).

2.5.2. Structure

PARP-1 has a modular organization comprising a N-terminal DNA-binding domain, which acts as a molecular nick sensor and contains two zinc-finger motifs called F1 and F2 that are involved in the recognition of DNA breaks during DNA repair (a third zinc finger termed F3 is not directly involved in the binding to DNA but is important when there is stimulation of the activity by the domain of DNA-binding [92]) and a bipartite NLS, a central regulating segment, which contains the automodification sites, and a BRCT (breast cancer suppressor protein 1 N-terminus) domain involved in proteineprotein interactions, and the C-terminal catalytic domain, which binds NAD^b. A domain of unknown function that carries the central motif WGR and is found in many polyA polymerases is located before the catalytic domain (Fig. 5). The determination of the 3-D structure of the catalytic domains of chicken PARP-1 and mouse PARP-2 showed that these proteins have structural homology with the active site of the bacteria ADP-ribosylating toxin of Corynebacterium diphteriae.

2.5.3. Related proteins

PARP, known today as PARP-1 (E.C. 2.4.2.30), is the founding member of a large PARP superfamily, which contains 17 members [93]. Some members might function together and possess overlapping properties. They contain a b-a-loop-b-a NAD^b fold, which is the most conserved region in PARP-1 orthologues and represents the so-called PARP signature (residues 859e908 of PARP-1). Some of these members lack crucial residues for poly(ADP-ribose) synthesis and show mono (ADP-ribosyl) transferase activity, some are even inactive enzymes [94].

2.5.4. Expression

PARP-1 is an abundant nuclear protein. It has many partners, i.e. histones [poly(ADP-ribosyl)ation of histones is associated with

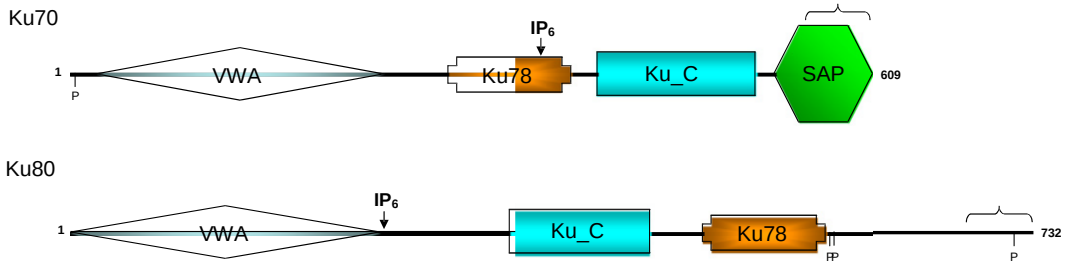

Fig. 4. Domain organization and epitopes of Ku70 and Ku80. The major B-cell epitopes of Ku70 and Ku80 (black parentheses) are located at the C-terminal ends. IP6, inositol hexakisphosphate binding site; Ku78, ATP-dependent helicase domain; Ku_C, Ku70/Ku80 C-terminal arm; P, phosphorylation site; SAP, DNA-binding domain; VWA, von Willebrand factor type A domain.

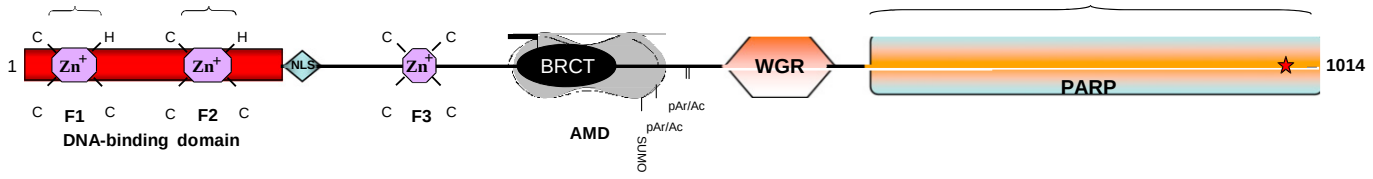

Fig. 5. Domain organization and epitopes of Poly(ADP-ribose) polymerase-1 (PARP-1). B-cell epitopes (black parentheses) are located in the catalytic PARP domain and in the N-terminal zinc fingers. AMD, automodification domain; BRCT, Breast cancer suppressor protein 1 N-terminus domain; NLS, Nuclear localization signal; pAr/AC, poly-ADP-ribosylation/acetylation sites; PARP, catalytic PARP domain; SUMO, sumoylation site; WGR, domain of unknown function containing the central motif WGR; Znþ, zinc-finger domain. The enzymatically active site at Glu988 is indicated by a red asterisk. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

open chromatin structure at the DNA-damage site], HMG proteins, topoisomerases I and II, DNA helicases, single-strand break repair and base-excision repair factors, and various transcription factors.

2.5.5. Location

PARP-1 is a nuclear protein, enriched in the nucleolus and present also at telomeres and centromeres.

2.5.6. Biological functions

PARP-1 uses NADþ as a substrate to synthesize a linear or multibranched polymer of ADP-ribose on glutamic acid, aspartic acid and lysine residues on various acceptor proteins, including itself. This drastic modification takes place in response to multiple cellular situations, including DNA damage, inflammation and cell-death induction. In addition to itself, PARP-1 modifies other proteins including histones H1 and H2B, DNA topoisomerases I and II, DNA polymerases, p53, various transcription factors, such as NF-kB, and proteins involved in DNA repair. PARP-2 preferentially heteromodifies H2B whereas PARP-1 preferentially modifies H1. Poly(ADP-ribosyl)ation reactions regulate not only cell-survival and cell-death programmes, but also an increasing number of other biological functions with which novel members of the PARP family have been associated. These functions include transcriptional regulation, telomere cohesion and mitotic spindle formation during cell division, intracellular trafficking and energy metabolism.

2.5.7. Knockout

PARP-1-deficient mice are viable. They are resistant to various types of inflammation including streptozotocin-induced diabetes and lipopolysaccharide-induced septic shock [95,96].

2.5.8. Modifications

PARP-1 undergoes automodification (poly-ADP-ribosylation) on lysine residues [97]. Another enzyme, poly(ADP-ribose) glycohydrolase (PARG) with endo- and exoglycolytic activities, cleaves glycosidic bonds between ADP-ribose units and generates free ADP-ribose. PARP-1 and PARG have concurrent actions that contribute to NADþ consumption in heavily damaged cells. PARP can also be phosphorylated, acetylated, ubiquitinylated and sumoylated. Sumoylation does not affect ADP-ribosylation but inhibits acetylation of PARP-1 revealing a complex crosstalk of post-translational modifications [98] (Fig. 5).

2.5.9. Autoantibodies

Abs reacting with whole or fragments of PARP-1 and with poly (ADP-ribose) have been detected in the serum of patients with systemic autoimmune diseases (notably SLE) and with Crohn's disease and ulcerative colitis, two autoimmune bowel diseases with a potential evolution towards colon cancer [99e102] (Table 5). Interestingly, it was shown that some subsets of anti-PARP IgG Abs inhibit PARP-1 activity [103] and delay apoptosis in living cells, probably by inhibiting caspase-3-mediated PARP-1 apoptotic cleavage by sterical hindrance [104].

2.5.9.1. Epitopes. B-cell epitopes recognized by IgG autoAbs from patients with SLE, MCTD, Crohn's disease and ulcerative colitis have been identified in the catalytic site of the enzyme as well as in the two zinc-fingers F1 and F2 [103,105] (Fig. 5).

2.5.9.2. Role in diseases. Abnormalities in PARP-1 activity have been shown in several autoimmune diseases, including SLE, Crohn's disease, ulcerative colitis, Graves's disease and Hashimoto thyroidis [106e108]. Although a number of physiopathological effects mediated through the PARP system has been observed in autoimmune diseases, and more specially in SLE, it is not known whether poly (ADP-ribosyl)ated proteins are specific targets for autoAbs. Since a rearrangement of histones was observed when nucleosomes are incubated with poly(ADP-ribose) chains [109], it may well be that following certain cellular events (and apoptosis for example), poly (ADP)ribosylation of histones within the nucleosome greatly affects their antigenicity and immunogenicity, and leads to an autoimmune response. Other substrates of PARP-1, such as DNA topoisomerase-I, a target of autoAbs in SSc, might also become immunogenic upon modification [106].

2.5.10. Autoreactive T cells

Unknown.

2.5.11. Therapy

Autoimmunity to PARP-1 has not been selected for therapeutic intervention so far. However, PARP inhibitors have promising pharmacological applications in potentializing the effect of anti-tumor drugs in cancer therapy as well as in the treatment of inflammatory, neurological and cardiac disorders.

2.6. High-mobility group box 1 protein (HMGB1)

HMGB1 (amphoterin) takes on a special position among the autoantigens highlighted in this review, since the protein's activity itself, more than autoantibodies or autoreactive T cells, is involved in exacerbating and sustaining autoimmune diseases. HMGB1 might constitute the classical damage associated molecular pattern (DAMP) that rings the alarm after sterile inflammation. The protein was named after its quick migration during electrophoresis, but is also extremely "mobile" in other biochemical aspects (e.g. only very short attachment to DNA site).

Table 5
Autoantibodies to whole PARP-1 and PARP F2 peptide.

	Whole PARP	PARP F2 peptide (aa 122e165)
SLE n ¼ 92	13%	54%
MCTD n ¼ 17	24%	88%
SSc n ¼ 19	0%	5%
pSS n ¼ 67	9%	42%
sSS n ¼ 16	3%	56%
Crohn's disease n ¼ 14	0%	86%
Ulcerative colitis n ¼ 12	0%	67%

From [101,105].

2.6.1. Size of autoantigen

215 aa (Molar mass 25 kD, but migrates at 30 kD in SDS-PAGE due to its high content of acidic and basic aa residues).

2.6.2. Structure

HMGB1 contains two L-shaped DNA-binding HMG-boxes (A and B) and an unstructured tail with 30 consecutive negatively charged aa residues that is responsible for intermolecular interactions, especially with HMG-boxes [110]. The HMG-boxes and the tail region are connected by a linker and a joiner region, respectively (Fig. 6).

2.6.3. Related proteins

The HMGB-proteins are encoded by 3 genes in mammals: HMGB1, HMGB2, and HMGB3. HMGB1 is an almost uniquely conserved protein across species (>99% similarity in mammals). Human HMGB2 and HMGB3 share 80% homology [111].

2.6.4. Expression

HMGB-proteins are present in all multi-cellular animals. In the body, HMGB1 is ubiquitous and abundant (>1 × 10^6 molecules per cell) [112]. Serum levels of secreted HMGB1 are below 5 ng/ml in healthy animals and humans but can be elevated up to 150 ng/ml in patients with sepsis, with the highest levels found in patients who died from sepsis [113].

2.6.5. Location

In the cell, the location of HMGB1 is mostly nuclear, bound to chromatin. However, the protein undergoes nucleus-cytoplasm shuttling upon secretion [114].

2.6.6. Biological functions

1) HMGB1 binds in the minor groove of chromosomal DNA without sequence specificity, thus bending DNA, promoting assembly of proteins such as p53, NF-κB, RAG1/2 proteins, steroid hormone receptors, and homeobox-containing proteins, and facilitating transcription, replication, and V(D)J recombination [115]. 2) HMGB1 is passively released in high amounts by necrotic cells and cells killed by cytotoxic lymphocytes, whereas release from apoptotic cells is scarce since HMGB1 is bound to chromatin remnants due to deacetylation of histone proteins during apoptosis [116]. However, recent studies suggest that late apoptotic cells (undergoing secondary necrosis) can also release significant amounts of HMGB1 [117]. In addition, HMGB1 is actively secreted by monocytes and macrophages via regulated exocytosis of secretory lysosomes in a second wave in response to TNFα, IL-1β, or LPS [113].

Extracellular HMGB1 (A) exerts mitogenic activity, attracting and activating stem cells [118], (B) promotes angiogenic activity that is linked to atherosclerosis progression [119,120], (C) increases permeability of endothelia and recruits plasminogen, thereby initiating a proteolytic process that enhances tissue penetration, (D) leads to Th1 cell polarization [121], (E) activates DCs and macrophages by maturating and mobilizing them [121,122], (F) acts as an endogenous immune adjuvant, enhancing the immunogenicity of apoptotic cells [122], and (G) has cytokine and chemoattractant function, causing in concert with other mediators a delayed and biphasic release of TNFα, thereby prolonging and sustaining inflammation [118–120].

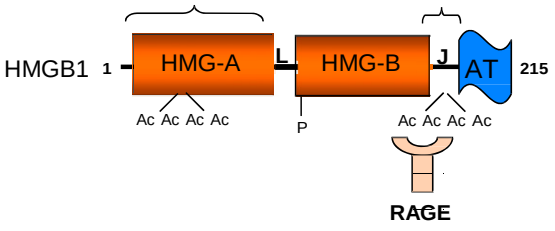

Fig. 6. Domain organization and epitopes of high-mobility group box 1 protein. AutoAbs from SLE patients appear to preferentially recognize residues in the box A (aa 1–76) and the J-region (aa 164–185) (black parentheses). Ac, acetylation site; AT, acidic tail; HMG-A and HMGB, DNA-binding HMG-boxes; L, linker region; J, joiner region; RAGE, receptor for advanced glycation end products-binding site.

2.6.7. Knockout

HMGB1 ko mice die shortly after birth [123].

2.6.8. Receptor

2.6.8.1. Receptor for advanced glycation end products (RAGE). HMGB1 recognizes S100 proteins, calgranulin, amyloid β-peptide in patients with Alzheimer, and advanced glycation end products [124]. It binds to RAGE via a motif present in the C-terminal region (aa 150–183) and leads to activation of NF-κB, Cdc42, and Rac (cell motility) [125,126]. HMGB1-RAGE interaction is important for sustaining NF-κB long-term activation in an autocrine/paracrine manner [124,127]. However, since RAGE knockout mice have a limited phenotype, RAGE is apparently not the only receptor for HMGB1 [118].

2.6.8.2. Toll-like receptors. There are conflicting results about an activation of TLR2 and TLR4 [128,129] by HMGB1. Furthermore, interaction with TLR9 enhances immunostimulatory effect of CpG ssDNA. RAGE ko cells respond poorly to CpG DNA [130,131]. Thus, HMGB1 might be necessary to activate TLR9 and also enhance immunogenicity of DNA-containing complexes such as nucleosomes.

2.6.8.3. CD24. Upon binding, CD24 negatively regulates the immunostimulatory capacity of HMGB1 by inhibition of NF-κB activation. This protects the host from lethal tissue damaged-induced immune responses [132].

2.6.9. Modifications

HMGB1 is heavily post-translationally modified. The modifications determine location and secretion of the protein. Secreted and released HMGB1 is acetylated on lysine residues throughout the length of the protein, particularly on segments 27–43 and 178–184, which act as nuclear localization signals (Fig. 6). The acetylation leads to a neutralization of positive charges within the nuclear localization signal and thus to a cytoplasmic relocation [133]. Furthermore, neutrophils monomethylate HMGB1 before secretion [134]. Recombinant (non-modified) HMGB1 is less active as an adjuvant, thus acetylation and/or monomethylation may go along with higher action of the protein or a variable potency in the activation of receptors, respectively [118]. HMGB1 is also subject to phosphorylation (Fig. 6).

2.6.10. Role of HMGB1 in diseases

2.6.10.1. Systemic lupus erythematosus. HMGB1 remains bound to nucleosomes released from late apoptotic cells in vitro. Such HMGB1-nucleosome complexes can be detected in the plasma from SLE patients. HMGB1/nucleosome complexes released from secondary necrotic cells under conditions of impaired clearance of apoptotic cells induce the secretion of cytokines, including IL-10, IL-1β, IL-6, and TNFα, as well as the expression of co-stimulatory molecules on macrophages and DCs, whereas HMGB1-free nucleosomes from neither viable nor apoptotic cells induce cytokine production or DC activation [135]. Furthermore, HMGB1-containing nucleosomes from apoptotic cells induced anti-dsDNA and anti-histone IgG responses in a TLR2-dependent manner, whereas nucleosomes from living cells did not. Thus, HMGB1-nucleosome complexes activate antigen-presenting cells and, thereby, may crucially contribute to the pathogenesis of SLE via breaking the immunological tolerance against nucleosomes/dsDNA.

2.6.10.2. Rheumatoid arthritis. Extracellular HMGB1 is abundantly expressed in the synovium and synovial fluid of RA patients and experimental arthritis models, whereas serum HMGB1 levels in RA do not correlate with disease activity [136–138]. In contrast to the strictly nuclear HMGB1 staining pattern observed in synovial cells collected from normal mice and rats, the increased expression in diseased animals occurs particularly in the cytosol of macrophage-like cells located in the sublining layer of the synovium, in vascular high endothelial cells and in certain synoviocytes in the lining layer and in lymphoid aggregates [137,138]. Aberrant expression precedes the onset of disease by five days. Intra-articular injection of HMGB1 induces synovitis in animals [139]. Prominent features of chronic synovitis are necrosis and activation of macrophages, both triggering the release of HMGB1 that stimulates synovial macrophages to release TNFa, IL-1, and IL-6 and enhances the activity of plasminogen activators, matrix mettaloproteiase (MMP-2), and MMP-9. HMGB1 therefore might have an important role in inflammatory and destructive processes of arthritis.

2.6.11. Autoantibodies

HMGB1 is a target antigen of perinuclear antineutrophil cytoplasmic antibodies (pANCA) that have been found in a high proportion of patients with autoimmune diseases [140–143] (see Table 6), as well as in MRL-lpr lupus mice [144].

2.6.11.1. Epitopes. Epitope mapping studies using sera from SLE patients revealed multiple epitopes spread over the whole molecule, but preferentially located within the HMG-A box and the J-region [145] (Fig. 6).

2.6.11.2. Role in diseases. Although the precise clinical role anti-HMGB1 autoAbs has not yet been elucidated, titers of these autoAbs are correlated with SLE Disease Activity Index (SLEDAI) scores and the number of platelets in lupus, and in RA with erythrocyte sedimentation rate, C-reactive protein, rheumatoid factor, joint score and hand grip strength [143,145]. Antibody levels decrease with clinical improvement. In contrast, the presence of anti-HMGB1 antibodies does not correlate with disease activity in PM/DM [145].

2.6.12. Autoreactive T cells

Pathogenic anti-DNA-autoAb inducing Th cell lines that are cross-reactive to HMGB1 were found in SLE patients [146].

2.6.13. Therapy

The A box of HMGB1 acts as an anti-inflammatory molecule that competitively inhibits the proinflammatory activities of full-length HMGB1 [147]. HMGB1's immune stimulatory effects such as attraction of inflammatory leukocytes after necrosis can also be blocked by HMGB1-Abs [116]. Application of anti-HMGB1 antibodies or A box reduces severity of established arthritis, weight loss and histological severity in collagen-induced arthritis [148] and is effective in the treatment of established sepsis [147]. The magnitude of the therapeutic effect is comparable with that of TNF-antagonists.

Table 6
Autoantibodies to HMGB1.

Systemic lupus erythematosus	45%
Polymyositis/Dermatomyositis	30%
Rheumatoid arthritis	16–48%
Juvenile idiopathic arthritis	39%
Systemic sclerosis	31–41%
Sjögren's syndrome	44%
Ulcerative colitis	32%
Autoimmune hepatitis	89%
Primary biliary cirrhosis	70%

3. RNA-associated autoantigens

3.1. The U1 snRNP complex

The U1 small nuclear ribonucleoprotein (snRNP) complex constitutes a major component of the spliceosome. U1 snRNP consists of a number of proteins (the seven Sm core proteins, B and its alternatively spliced variant B^{0}, D1, D2, D3, E, F, G, and the U1 snRNP-specific proteins U1-70, U1-A, and U1-C) that are either directly or indirectly associated with the small non-coding U1 snRNA. SnRNPs bind themselves to newly synthesized pre-mRNA and are essential for the removal of introns. SnRNPs are the prototype target of a directly pathogenic autoimmune response in SLE and MCTD.

3.1.1. Size of protein autoantigens

U1-70K (RNP 70): 437 aa (51.6 kD); U1-A (RNP A): 282 aa (31.3 kD); U1-C (RNP C): 159 aa (17.4 kD); Sm B: 231 aa (23.7 kD); Sm B^{0} (splice variant of Sm B): 240 aa (24.6 kD); Sm D1: 119 aa (13.3 kD); Sm D2: 118 aa (13.5 kD); Sm D3: 126 aa (13.9 kD); Sm E: 84 aa (10.8 kD); Sm F: 86 aa (9.7 kD); Sm G: (76 aa (8.5 kD).

3.1.2. Structure

The 165-nucleotide U1 snRNA forms a characteristic secondary structure consisting of four stem loops and a connecting helix and possesses multiple regions of dsRNA repeats [149]. U1 RNA shares a common secondary structure across species although the length of the RNA can vary significantly [150]. The U1-70K and U1-A proteins bind directly to the U1 snRNA via their RRM (one RRM in RNP 70, two in RNP A), whereas U1-C associates via protein–protein interaction with U1-70K and the Sm proteins [151]. RNP C contains an U1-like zink finger common for RNA-binding proteins [152]. The Sm proteins form a heptamer ring (~20 nm diameter) with the U-rich part of the U1 RNA passing through the center [153]. Common to the Sm proteins is the presence of one Sm-domain that assembles with the U1, U2, U4/U6 and U5 RNAs [152] (Fig. 7).

3.1.3. Related proteins

Other snRNPs of the spliceosome include U2, U4/U6, U5, and the less abundant U11, U12 and U4atac. These complexes share the same core structure (i.e. Sm) but also contain specific proteins, some of which are however related (e.g. U2-B^{00}, U2-A^{0}).

3.1.4. Expression

The U1 snRNA is with its 10^{6} copies/nucleus highly abundant in the nucleus [154].

3.1.5. Location

Nuclear. Translocation to locations where it could encounter TLRs occurs in dead and dying cells [155].

3.1.6. Biological function

The U1 snRNP is a major component of the spliceosomal machinery that catalyzes splicing of pre-mRNA into mature translatable mRNA. The RNA in these particles is like ribosomal RNA in that it has both an enzymatic and a structural role.

3.1.7. Knockout

The deletion of the Sm D1 gene is lethal in yeast [156], whereas U1-A deficient Caenorhabditis elegans are viable due to functional redundancy of U1-A with the related protein U2-B^{00} [157].

3.1.8. Modifications

During apoptosis U1-70K is specifically cleaved by caspase-3, converting it into a C-terminally truncated 40 kD protein that

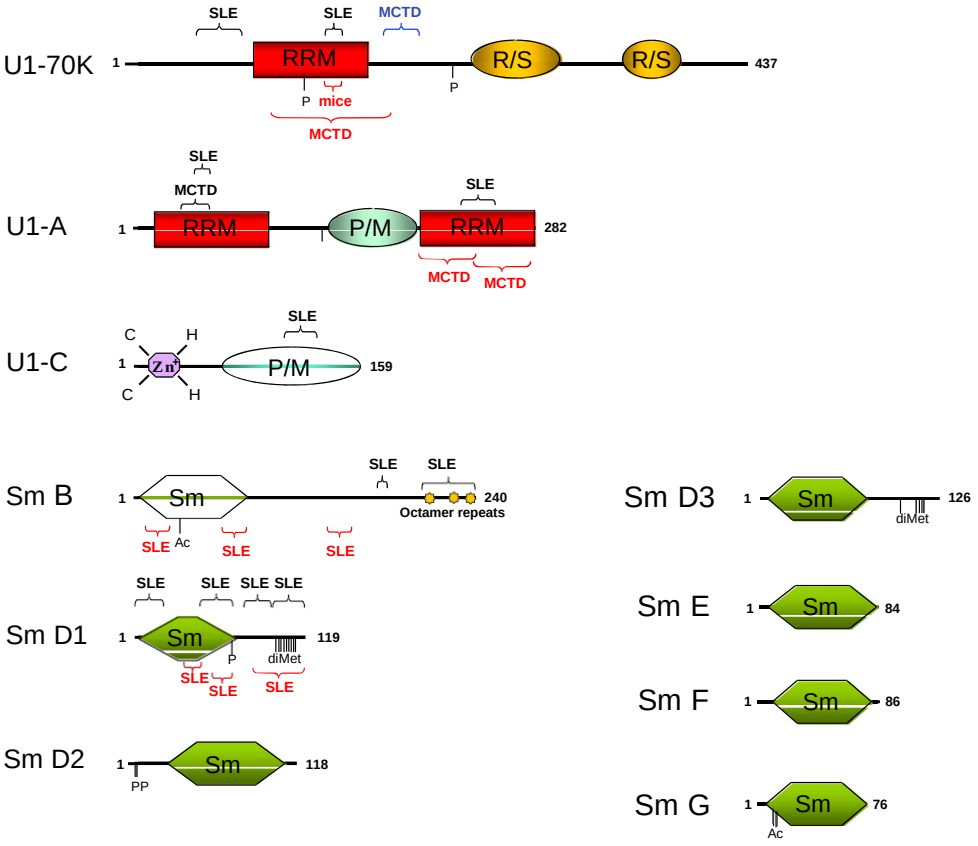

Fig. 7. Domain organization and epitopes of components of the U1 snRNP complex. Both B and T-cell epitopes have been identified for the major autoantigens of the U1 snRNP complex, U1-70K and U1-A as well for the Sm core proteins Sm D1 and Sm B. Major B-cell epitopes are depicted by black parentheses, T-cell epitopes by red parentheses, an apotope recognized by MCTD sera is depicted by a blue parenthesis. Ac, acetylation site; diMet, dimethylation site; P, phosphorylation site; P/M, proline/methionine-rich region; RRM, conserved RNA-recognition motif; R/S, arginine/serine-rich region; Sm, Sm-domain; Znþ, zinc-finger-like region. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

remains associated with the U1 RNA [158,159]. Before cleavage by caspase-3, the U1-70K protein undergoes a specific change in its phosphorylation state, consisting of the phosphorylation of Ser^{140} in the RRM and the dephosphorylation of other serine residues in a process that is caspase-dependent and PP1 phosphatase-mediated [160]. This hypophosphorylated protein is then clustered into so-called heterogeneous ectopic RNP-derived structures (HERDS) and is finally released into apoptotic bodies. The altered localization of the U1 complex in apoptotic cells, together with post-translational modifications may be critical determinants in the development of an immune response in susceptible individuals [161]. B and T cell immunity to apoptotically modified proteins can develop against caspase-cleaved forms or against forms that undergo additional post-translational modification [160,162,163]. The U1 RNA looses the 2,2,7-trimethylguanosine cap during anti-Fas induced apoptosis, which however does not affect stability of the complex [159]. Sm B, Sm D1 and Sm D3 undergo dimethylations in the methylosome [164] (Fig. 7).

3.1.9. Autoantibodies

Approximately 10% of lupus patients have autoAbs to Sm proteins, up to 30% have anti-RNP Abs (directed against U1-A, U1-C, and U1-70 kD) [165] (Table 7). Anti-Sm/RNP-positive autoimmune sera show a typical speckled pattern in the classical Hep2 staining [166]. The prevalence of anti-Sm Abs in MRL-lpr mice is ~25% [167]. AutoAbs to Sm proteins are also present in the sera from (NZB × NZW)F1 mice [168]. A part of sera from patients with MCTD specifically recognize the apoptotic form of U1-70K, which displays (an) epitope(s) that is/are not present on the intact form of the protein [169] (Fig. 7). AutoAbs directed against this apotope appear to be more specific for MCTD than other anti-U1-70K autoAbs and decrease in titer during the progression of MCTD [170].

3.1.9.1. Epitopes. The major B-cell epitopes for U1-70K and U1-A are located within RNA-binding domains [152,171,172]. On Sm B, Sm D1 and Sm D3 the dimethylarginine-modified C-terminal RG repeats constitute major B-cell epitopes that could also be responsible for cross-reactions between Sm proteins [173]. The major epitope of U1-C contains the proline-rich sequence APGMRPP (119e125 aa) [174]. A similar epitope is also present in U1-A and Sm B/B^0 and can be cross-recognized by autoAbs targeting them [175] (Fig. 7). These motifs might be involved in the initiation of B-T cell spreading [171,176].

3.1.9.2. Role in diseases. U1 snRNP but also U1 RNA alone can induce the secretion of proinflammatory cytokines such as IL-6 and IL-8 [149]. TLR7 is specifically required for generation of autoAbs directed to the

Table 7
Autoantibodies to components of the U1 snRNP.

	Anti-Sm	Anti-U1 RNP
SLE	10e30%	30e40%
MCTD	0%	100%[a]
SSc		10e15%

[a] By definition, 100% of MCTD patients possess anti-U1 snRNP Abs since it is a criterium for diagnosis.

RNA component in snRNPs and the development of glomerulonephritis in pristane-induced murine lupus [177]. Sm/RNP is taken up via the B cell-receptor and is activating TLR subsequently [166]. The U1 snRNA component of U1snRNP immune complexes, found in patients with SLE, acts as an endogenous "self" ligand for TLR7 [178]. Furthermore, recognition of apoptotically and oxidatively modified forms of the U1-70K autoantigen have been reported to be associated with distinct clinical disease manifestations [179].

In SLE, autoAbs directed to components of snRNPs, specifically U1 snRNPs, are the major autoantigens apart from nucleosomes and dsDNA [178]. They correlate with an activated IFNa-pathway and with lupus severity, including glomerulonephritis [180]. SnRNP-containing immune complexes activate autoreactive RF-positive B cells by involvement of TLR7 and the B cell-receptor [166]. Mammalian U1 snRNP particles activate human pDCs resulting in strong TLR7-dependent IFNa-responses [181,182] and together with autoantibody-positive sera stimulate IFNa-production via CD32 (FcgRII) [182].

In MCDT, anti-RNP antibodies have been found to interact with lung tissue in ways that could mediate MCTD lung pathology [183]. Immunization of susceptible mice with RNP antigen could induce MCTD-like serologic responses and some clinical manifestations [169]. Anti-U1 RNP autoAbs appear to directly interact with the splicing process and lead to the generation of a MCTD-associated isoform of angiopoietin [184]. Furthermore, immunization of BL/6 DR4-transgenic mice with U1-70K or U1 RNA has been reported to lead to MCDT-like lung disease with autoAbs correlated to severity [185].

3.1.10. Autoreactive T cells

A T-cell epitope located in the RNP1 motif present within the RRM of the U1-70K protein has been characterized using T cells from patients with lupus and MRL-lpr and (NZB × NZW)F1 mice [168,186,187]. T cells from MCTD patients recognize five distinct epitopes on U1-70K that are also located within the RNA-binding domain [188]. Furthermore, T-cell epitopes have been identified in the U1-A protein [189,190] and SmD1 protein [189,191]. Intramolecular T-cell epitope spreading occurs in lupus-prone MRL-lpr mice, and the region 131e151 of the U1-70K protein is important in the cascade of events observed in the murine lupus autoimmune response [192] (Fig. 7).

3.1.11. Therapy

The phosphorylated peptide 131e151 of the U1-70K protein called P140 is recognized by T cells from NZB/W and MRL-lpr mice as well as by T cells from patients with SLE, and intravenous administration of the peptide into MRL-lpr mice was found to significantly improve their clinical and biological manifestations and to prolong their survival [163,186]. In a phase I and II clinical trial conducted by ImmuPharma (Mulhouse, France), the P140 peptide (lupuzor™) was found to be safe and well-tolerated by test subjects. It led to a significant decrease of anti-DNA antibodies and significantly improved the SLE Disease Activity Index (SLEDAI) score and the biological status of lupus patients [193]. P140 peptide is currently evaluated in a phase IIb, double-blind, placebo-controlled, dose-ranging study in Europe and Latin America (Cephalon, Frazer, PA).

The cognate peptide sequence contains a conserved RNP1 motif that is also found in other RNP and non-RNP proteins. Autoreactive T cells to this candidate sequence might drive an early diversification of the autoimmune response in Lupus. Treatment with the phosphorylated peptide might originate a mechanism of tolerance spreading that leads to the beneficial effect observed in MRL-lpr mice [192] and patients. The P140 peptide might control the disease in inducing apoptosis of activated lymphocyte subpopulations occurring in lupus [194].

3.2. The U3 snoRNP complex

Autoantibodies to the U3 small nucleolar ribonucleoprotein (snoRNP) occur in 8e10% of patients with diffuse cutaneous SSc and are mainly directed to fibrillarin, the enzymatic component of the U3 snoRNP particle that shows methyltransferase activity. Fibrillarin is thought to participate in the first step of processing of preribosomal RNA. In humans, fibrillarin is associated with the U3, U8, and U13 small nucleolar RNAs.

3.2.1. Size of the autoantigen

320 aa (36 kD).

3.2.2. Structure

Fibrillarin is composed of an N-terminal Gly/Arg-rich region containing an NLS, a central RNA-binding domain that includes an RNP-2-like consensus sequence, and a C-terminal a-helical domain responsible for targeting to Cajal bodies [195,196] (Fig. 8).

3.2.3. Related proteins and evolutionary conservation

Despite their structural similarity, methyl-transferases such as fibrillarin display little overall sequence homology across different functional classes. All of them share, however, conserved regions that are responsible for binding of S-adenosyl-1-methionine and support the catalytic reaction [197].

3.2.4. Expression

Fibrillarin is universally present in eukaryotes and shows a striking degree of evolutionary conservation of primary structure. Functional homologs exist in Archaea but not in bacteria.

3.2.5. Location

Nucleolus.

3.2.6. Biological function

SnoRNPs are involved in the processing of pre-rRNAs. They are composed of a set of proteins that is required for the enzymatic activity, stability and nucleolar localization of the snoRNPs, and small nucleolar guide RNAs (snoRNAs). Fibrillarin is the catalytic subunit of the C/D class of snoRNPs and carries out $2^{\prime}$-O-methylation of rRNAs. It is assumed that methylation and pseudouridylation facilitates the folding and increases the stability of rRNA [198].

3.2.7. Knockout

No knockouts exist in higher eukaryotes but the protein was shown to be essential for viability in yeast and C. elegans [195].

3.2.8. Associated nucleic acid

Anti-fibrillarin Abs (AFA) precipitate a number of small nucleolar RNAs, the most abundant being U3 RNA.

3.2.9. Modifications

Fibrillarin is dimethylated at arginines residues in the N-terminal GAR-region [199] (Fig. 8).

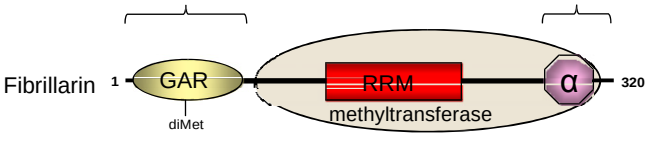

Fig. 8. Domain organization and epitopes of fibrillarin. The conformational major B-cell epitope of fibrillarin comprises N- and C-terminal parts of the protein (black parentheses). The GAR-region contains clusters of glycine and methylarginine/dimethylarginine-residues. a, C-terminal alpha-helical region; diMet, dimethylation site; GAR, glycine- and arginine-rich region; RRM, RNA-recognition motif.

Table 8
Autoantibodies to fibrillarin.

Systemic sclerosis	
AfricaneAmerican	16e43%
Caucasian	4e5%
Males	8e33%
Females	7e14%
Diffuse cutaneous SSc	9%
Limited cutaneous SSc	4%
Autoimmune myositis	14%

3.2.10. Autoantibodies

Abs to U3 snoRNP are highly specific SSc, and are found more frequently in blacks, males and patients with a younger age at disease onset [200,201] (Table 8). There is a strong association with certain MHC class II haplotypes (DRBZ *Z302, DQBZ*0604) [201].

3.2.10.1. Epitopes. Although a number of studies have attempted to establish a cartography of epitopes the exact binding site of AFA remains unknown. It has been suggested that the major epitope(s) is (are) conformational in nature and comprise(s) N- and C-terminal structures [202].

3.2.10.2. Pathogenic relevance. Association has been described with skeletal muscle disease, primary pulmonary arterial hypertension, gastrointestinal dysmotility, diffuse skin involvement, extensive telangiectasias and heart and lung involvement [200,203]. Certain mouse strains develop AFA following exposure to mercury or silver. Histopathologic analyses also have demonstrated renal and vascular lesions in these animals, suggesting that AFA may be pathogenic.

3.2.11. Autoreactive T cells

Unknown.

3.2.12. Therapy

Autoimmunity to fibrillarin has not been selected for therapeutic intervention so far.

3.3. Heterogeneous nuclear A/B ribonucleoproteins (hnRNP-A/B)

Heterogeneous nuclear ribonucleoproteins (hnRNPs) are a group of multifunctional RNA-binding proteins shuttling between the nucleus and cytoplasm of mammalian cells. Proteins of hnRNP complexes have been first described as autoimmune targets already 20 years ago Most prominent targets are the closely related hnRNP-A1 and -A2 proteins and their splice variants, the hnRNP-B proteins; together with hnRNP-A3 and hnRNP-D proteins they form the subgroup of hnRNP-A/B proteins. They are recognized by autoantibodies in rheumatoid arthritis, systemic lupus erythematosus, mixed-connective tissue disease, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), and multiple sclerosis (MS) patients, as well as in animal arthritis and lupus models.

3.3.1. Size of autoantigens

hnRNP-A1: 320 aa (w34 kD), splice variant A1b (372 aa); hnRNP-A2: 340 aa. Molar mass 36 kD, migrates at 33 kD in SDS-PAGE (therefore named RA33 after its discovery as target of autoAbs in patients with RA); hnRNP-B1 (splice variant of -A2): 352 aa (37 kD); hnRNP-A3: 378 aa (four isoforms, 38e41.5 kD); hnRNP-D (AUF1): 355 aa (37 kD).

3.3.2. Structure

The typical structure of the family includes a number of adjacent N-terminal RNA-recognition motifs (RRM) and a glycine-rich C-terminal part containing an RGG box that is responsible for intra- and intermolecular interactions. A transportin-binding nuclear localization signal (M9) is thought to be important for shuttling between the nucleus and cytoplasm (see Fig. 9). HnRNPs-A1 and -A2 bind a large variety of RNA- and DNA sequences (reviewed in [204]).

3.3.3. Related proteins and evolutionary conservation

The family of hnRNPs consists of about 30 evolutionary conserved proteins that associate with nascent pre-mRNA [205]. Among these, the hnRNP-A/B proteins form a subgroup of highly related proteins. The three A-proteins are encoded by different genes, while the B-proteins A1b and B1 represent splice variants of A1 and A2, respectively. The hnRNP-D proteins are a subgroup of four proteins generated by alternative splicing that are also known as AU-rich element binding factor 1 (AUF1) and show the same general structure, but have an extra N-terminal domain that is unique to this group and essential for its function [206].

3.3.4. Expression

HnRNP-A1 and hnRNP-A2 constitute 60% of the total protein mass of hnRNP particles, representing the most abundant nuclear proteins (HnRNP-A1 is present in $7e10 \times 10^7$ copies per HeLa cell) [207]. HnRNP-A2/B1 is expressed in many organs, with the highest expression seen in the skin, lymphoid tissue, brain, and reproductive organs. There is also a particularly high expression of hnRNP-A/B proteins in inflamed synovial tissue and in lung cancer and the proteins appear to have a specific function upon stress/inflammation.

3.3.5. Location

HnRNP-A/B proteins show a primarily nuclear location but are able to shuttle between the nucleus and the cytoplasm where they are involved in the regulation of translation. Abundant cytoplasmic localization has been reported in inflamed synovial tissue. HnRNP-A/B proteins have also been found at the external side of plasma membranes and in extracellular fluid.

3.3.6. Biological function

HnRNPs are involved in pathways along mRNA biosynthesis, including pre-mRNA splicing, transport of mRNA and regulation. HnRNP-D (AUF1) is a part of the mRNA decay complex that binds to uridine- and/or adenine-rich regions in the 3^0untranslated region of mRNAs such as c-fos or TNFa, and leads to destabilization and rapid degradation of the mRNA [206,208].

3.3.7. Knockout

No knockouts are known for hnRNP-A1, -A2. HnRNP-D ko mice develop normally but suffer from an inability to degrade mRNA for inflammatory cytokines leading to high susceptibility for lethal endotoxemia [209].

3.3.8. Modifications

Most of the hnRNPs are phosphorylated and/or methylated in vivo. Dimethylation at arginine residues affects their binding to nucleic acids [210] (Fig. 9).

3.3.9. Autoantibodies

AutoAbs to hnRNP-A2/B1 occur in about 30% of RA, SLE, and MCTD patients and may rarely cross-react with hnRNP-A1. AutoAbs to hnRNP-A1 can also be found in RA/SLE/MCTD, but most autoAbs recognizing hnRNP-A1 are probably in fact cross-reacting anti-hnRNP-A2 Abs. AutoAbs to the hnRNP-C proteins were occasionally found in the sera of patients with SSc, psoriatic arthritis, and myositis [211]. AutoAbs to hnRNP-D have been reported to occur in 33% of SLE, 20% of RA, and 17% of MCTD patients [212]. HnRNP-I is an autoantigen in SSc [213]. Anti-HnRNP-L autoAbs are found in association

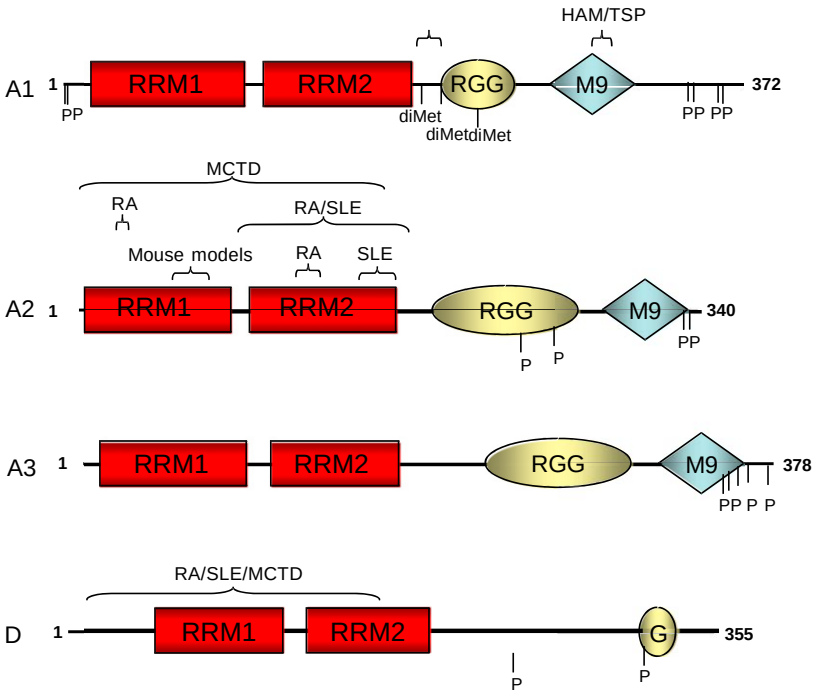

Fig. 9. Domain organization and epitopes of the heterogeneous nuclear ribonucleoproteins A1, A2, A3 and D/AUF1. Major B-cell epitopes identified in hnRNP-A2 and hnRNP-D/AUF1 are depicted by black parentheses. diMet, dimethylation site; G, glycine-rich region; M9, Nuclear localization signal; P, phosphorylation site; RGG, Arginine and glycine-rich region; RRM, conserved RNA-recognition motif.

with hnRNP-A/B autoAbs [214]. AutoAbs to hnRNP-A1/A2/B1 were detected in the cerebrospinal fluid (but not in the sera) of 91% of patients with MS [215], and hnRNP-A1 Abs are found in cerebrospinal fluid and in sera of patients with HAM/TSP [216] (Table 9).

3.3.9.1. Epitopes. In RA, Abs to conformational B-cell epitope(s) are located within the hnRNP-A2 region 87e182 [217]. A conformational A2-epitope recognized by Ab from MCTD patients comprises both RRMs and appears different from another one recognized by Abs from SLE and RA patients [217]. Short linear epitopes appear to be selectively recognized by autoAbs from SLE patients and recognition of one particular epitope was found to correlate with lupus disease activity [218]. The epitopes involved in HAM/TSP are encompassed in hnRNP-A1 191e202 and 293e304, and HTLV-1-tax 346e353 [219]. The main B-cell epitope recognized by Abs in animal models seems to be hnRNP-A2 50e70 [220,221] (Fig. 9).

3.3.9.2. Pathogenic relevance. The B-cell epitope hnRNP-A2 155e175 appears to be specific for SLE and is significantly associated with disease activity, skin rash, renal and mucocutaneous manifestations, and arthritis in lupus [218]. In contrast to SLE, anti-RA33 Abs in RA may be associated with a more benign disease [222] However, B and T cell reactivity to some linear sequences seems to be linked to active disease and erosion in arthritis ([218] and unpublished results from our lab). Abs against hnRNP-A1 cross-react with the HTLV-1-tax protein, thus suggesting molecular mimicry between the two proteins. Monoclonal Abs against hnRNP-A1 of HTLV-1-tax completely inhibited neuronal firing, indicating a potential pathogenic role in HAM/TSP [223]. Spontaneously produced Abs against hnRNP-A2 occur in mouse models of lupus (MRL-lpr mice [221]) and in mouse models of RA (hTNFtg mice, [220]). HnRNP-A2 appears also to be a primary autoantigen in pristane-induced arthritis [224] and splenocytes restimulated with hnRNP-A2 can transfer arthritic disease (our unpublished data), thus strengthening the pathogenic role of this autoantigen.

3.3.10. Autoreactive T cells

T cells reacting to hnRNP-A2 were found in the peripheral blood of about 60% of RA patients [225], as well as in 66% of SLE patients [226]. 15% of RA patients had T cells recognizing specific hnRNP-A2 peptides [unpublished data]. Furthermore, T-cell clones have been isolated from patients with SLE and MCTD [227].

3.3.11. Therapy

Nasal vaccination with whole protein hnRNP-A2 in saline has shown promising results in pristane- and glucose-6-phosphate isomerase (GPI)-induced arthritis (our unpublished results).

3.4. The Ro/La complex

Antibodies to the Ro antigen were first reported in 1962 in sera from patients with primary Sjögren's syndrome [228]. The antigen is therefore also known as Sjögren's syndrome antigen A (SS-A). The main autoantigen of the Ro-complex, Ro60, is complexed with one of four small non-coding RNAs called Y RNAs. In human and other vertebrate cells, a large fraction of the Ro60/Y RNA complexes also contains the La protein, a nuclear phosphoprotein that binds many newly transcribed non-coding RNAs, including Y RNAs. The La protein was originally defined by its reactivity with autoantibodies

Table 9
Autoantibodies to hnRNP-A/B and -D.

Rheumatoid arthritis	hnRNP-A2/B1	30%
	hnRNP-D	20%
Systemic lupus erythematosus	hnRNP-A2/B1	30%
	hnRNP-D	30%
Systemic sclerosis	hnRNP-I	55%
Mixed-connective tissue disease	hnRNP-A2/B1	35%
	hnRNP-D	17%
Multiple sclerosis	hnRNP-A1/A2/B1	91%
HTLV-1-ass. myelopathy/tropical spastic paraparesis	hnRNP-A1	100%

from patients with Sjögren's syndrome and SLE (the name is derived from the patient in whom the autoantibody was detected first) and is now also known as Sjögren's syndrome antigen B (SS-B). Today evidence is accumulating that autoimmunity to the Ro/La RNP is directly involved in pathogenic events in humans, especially in the heart disease of neonatal lupus.

3.4.1. Size of the autoantigens

Ro/SS-A: 538 aa (60 kD); La/SS-B: 408 aa (50 kD).

3.4.2. Structure

Structural analysis of Ro60 performed in Xenopus (78% sequence identity with human Ro60) revealed a major domain (aa 30e360) consisting of a series of alpha-helical repeats, known as HEAT repeats, that are arranged to form a doughnut with an inner hole with a diameter of 1e1.5 nm, wide enough to accommodate single-stranded, but not double-stranded RNA. The ring of HEAT repeats is clasped shut by a second domain, which resembles the von Willebrand Factor A (VWA) domain found in a number of extracellular matrix proteins and in proteins that function in cell adhesion. Y RNAs are thought to bind to conserved residues on the outside of the doughnut. The only other RNA species found to associate with Ro60 are misfolded small non-coding RNA such as 5S rRNA or U2 RNA. The 3^{0} ends of such misfolded RNAs bind inside the central hole of the Ro60 doughnut while helical portions of these RNAs bind to surfaces overlapping the Y RNA-binding site [229]. Since Y RNA binding would sterically block misfolded RNAs from binding, it has been suggested that one role of Y RNAs might be to regulate access of Ro60 to other RNAs. However, there is to date not much evidence that such function is of relevance in vivo.

La comprises a conserved so-called La motif at the N-terminus (aa 1e60) [230] that has been suggested to fold into a RNA-binding motif [231], a lesser conserved RRM-like domain [232] and a weakly conserved C-terminus [233]. La is eventually able to form homodimers, and dimerization is mediated by sequences in the C-terminal region [234] (Fig. 10).

3.4.3. Related proteins

In addition to the authentic La protein, all sequenced eukaryotic genomes contain one or more La motif-containing proteins which are otherwise not related to the La antigen [233]. Most patients with anti-Ro60 autoAbs also produce Abs against a structurally unrelated 52 kD E3-ligase termed Ro52 (also known as TRIM21) [235]. Ro52 possesses multiple N-terminal zinc-finger motifs, a central leucine zipper, a potential N-glycosylation site [236], but does not bind to nucleic acids. It remains questionable if Ro52 is physically associated with the Ro60/Y RNA complex in the cell [237,238]. However, Ro52 and the chaperone calreticulin appear to colocalize with Ro60 in apoptotic blebs [239]. Furthermore, autoAbs to Ro60 (and to Sm) cross-react to EpsteineBarr virus nuclear antigen 1 (EBNA-1) [240]. Therefore it is hypothesized that the autoimmune initiating event in human lupus could be an Ab that arises from the EBNA-1 response and cross-reacts with lupus autoantigens.

3.4.4. Expression

Ro60 is found in many if not all animal cells, but not in yeast. Interestingly however, orthologues have been described in the green algae Chlamydomonas and the eubacterium Deinococcus radiodurans [241].

La is ubiquitous in eukaryotic cells and with about 2×10^{7} copies per cell approximately 50 times more abundant than Ro60 [233], reaching concentrations of 50 nM in human S100 extract [242] and 12 nM in S. cerevisiae extract [243], respectively.

3.4.5. Location

Ro60 and La are mainly confined to the nucleus in normal cells, but a portion of it can be detected in the cytoplasm indicating that the proteins have also a cytoplasmic function. Similarly, the La protein becomes cytoplasmic during apoptosis of mammalian cells or poliovirus infection [239,244e246].

3.4.6. Biological function

La is involved in diverse aspects of RNA metabolism such as binding and protecting 3^{0} UUU(OH) elements of newly transcribed

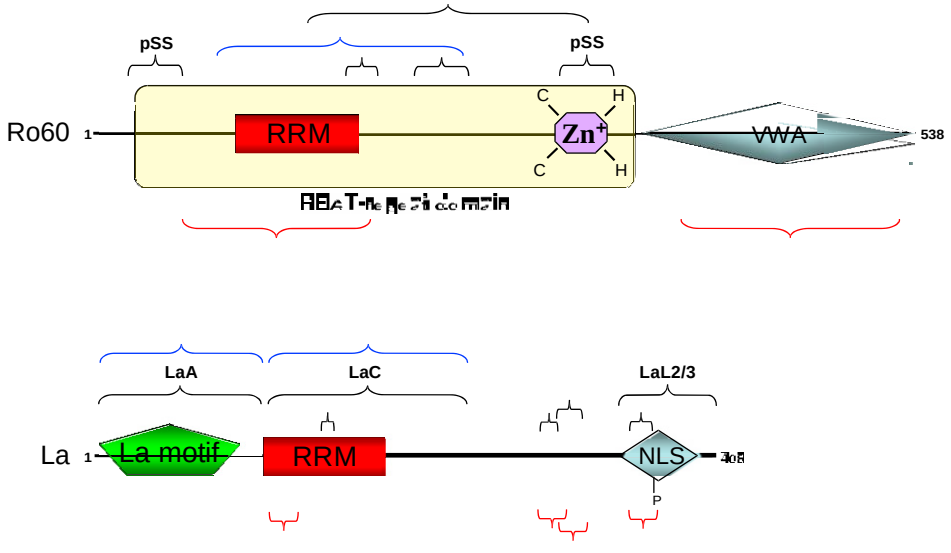

Fig. 10. Domain organization and epitopes of Ro60 and La. For the La autoantigen three immunodominant epitopes have been identified: LaA (aa 1e107), LaC containing the RNA-recognition motif (aa 111e242), and LaL2/3 (aa 346e408). LaA and LaC are also exposed as apotopes while LaL2/3 is masked, presumably by maintaining an intracellular location. The immunodominant B-cell epitope of Ro60 spans aa 140e325, an immunodominant apotope in Ro60 has been mapped to aa 82e244. Several minor linear epitopes of Ro60 and La do exist as well. B cell autoantigenic regions are depicted by black parentheses, murine T-cell epitopes by red parentheses, apotopes by blue parentheses. NLS, nuclear localization signal; P, phosphorylation site; RRM, RNA-recognition motif; Znþ, zink finger domain. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

RNA, processing 5′ and 3′ ends of pre-tRNA precursors, and acting as an RNA chaperone since transient binding by the La protein facilitates the correct folding of many newly synthesized small RNAs [247,248] and protects them from exonuclease digestion [233]. For many small RNAs the binding by La contributes to nuclear retention of the nascent transcripts [249,250] and/or assembly of the RNAs into functional RNPs [248,251]. As the binding sites for La are the RNA's terminal uridylate residues that are normally removed during maturation of RNAs, La binds normally RNA precursors rather than mature RNAs.

The exact biological role of Ro60 has still not been fully elucidated, but it has been suggested that Ro60 binds misfolded, defective non-coding RNAs that are eventually degraded, thus working in a quality control system that marks damaged RNAs for decay [252,253] Ro60 seems also to play a role in cellular resistance to UV irradiation [254], probably by being involved in the degradation of damaged RNAs following UV irradiation.

The function of Y RNAs may be to block access of the central cavity of Ro60 to other RNAs [229]. Most of these Y RNAs are bound to the Ro60 protein both in the nucleus and in the cytoplasm [253,255]. In addition, at least a fraction of the hY RNAs is also bound by the La protein which contributes to nuclear retention of Y RNAs [249,250,256].

3.4.7. Associated nucleic acids

In contrast to Ro60 which appears to mainly associate with Y RNAs, La is associated with a large number of nascent small RNAs, pre-tRNAs, pre-5S rRNA, U6 snRNA, RNase P RNA, MRP RNA, 7SL RNA, Y RNAs, rodent 4.5SI and 4.5SII RNAs, and transcripts of Alu sequences, but also virally-encoded RNAs. As in yeast, La may bind newly synthesized pre-U1 RNAs in vertebrate cells. However, precursors to U2, U4, and U5 snRNAs have not yet been described as associating with the La protein in metazoan cells [233].

3.4.8. Knockout

Mice lacking Ro60 have very low levels of Y RNAs but develop normally. However, they show increased sensitivity to UV irradiation and may generate autoAbs against chromatin and ribosomes and subsequently develop a lupus-like syndrome with glomerulonephritis and increased photosensitivity [257]. Despite these findings the role of Ro60 and Y RNAs, respectively, in regulation of the immune response is far from being understood since no follow-up studies have been published so far.

La is essential for snRNP biosynthesis in yeast strains in which Sm proteins are mutated. In these cells, La becomes required for incorporation of the U4 RNA during U4/U6 snRNP assembly [251]. This is presumably also the case in vertebrate cells [233].

3.4.9. Modifications

La proteins from human to yeast are phosphorylated in vivo, the phosphorylation sites being located within the C-terminal region [230,258] (Fig. 10). La has been shown to undergo proteolytic cleavage during apoptosis resulting in the loss of the NLS and accumulation in the cytoplasm [244] whereas Ro60 is not cleaved during cell death [259]. Ro and La polypeptides that are redistributed to membrane blebs in apoptotic keratinocytes may present neo-epitopes via molecular modifications such as oxidation, proteolytic cleavage or conformational changes [259,260]. Ro60 translocates to the surface of early apoptotic cells and remains immunoreactive on the late population, whereas La is present in apoptotic blebs only during late apoptosis. Cell surface topology studies performed for La revealed that during apoptosis the NH_2-terminal and central regions are extracellularly exposed and accessible to circulating antibodies whereas the COOH-terminus remains intracellular [261].

3.4.10. Autoantibodies

Anti-Ro60 autoAbs occur in 38–90% of patients with SS, 24–60% of patients with SLE and in 70–100% of patients with subacute cutaneous lupus erythematosus (SCLE) [262] (Table 10). The presence of anti-Ro60 Abs is one criterion used in the diagnosis of pSS [263]. Most patients with Abs to the Ro60 protein also produce Abs against the structurally unrelated Ro52 protein [235]. Maternal antibodies to Ro60, Ro52 and La are also highly associated with neonatal lupus [262,264].

About half of patients with autoAbs against Ro also have autoAbs against La (25–50% of patients with SS, 10–15% of patients with SLE, and 60% of mothers of children with neonatal Lupus). This suggests that the Ro/La ribonucleoprotein is the autoantigenic particle, rather than one of the individual Ro proteins [262]. The spreading of the immune response from Ro to La is thought to arise by intermolecular B-T cell help after initiation of T cell autoimmunity to one component of the Ro/La RNP, most likely Ro60 [265]. Thus, autoreactive T cells primed to a Ro60 determinant might provide help to both Ro60 and La specific B cells. B cells of either specificity could subsequently present Ro60 determinants to Ro60-specific T cells with production of anti-Ro60 and anti-La autoAbs.

3.4.10.1. Epitopes.

At least one conformational major epitope region exists within the middle third of the Ro60 protein, spanning roughly amino acids 140–325 (recognized by 80% of Ro-reactive sera). This portion of the protein contains all the residues that contact the Y RNA fragment in the crystal structure, as well as the residues in the inner hole that likely contact the 3′ ends of misfolded RNAs [229]. Significant numbers of patients' sera also recognize minor linear determinants outside this region [266–268] (Fig. 10). Some of them are preferentially recognized by Abs from patients with pSS indicating that different mechanisms are involved in the immune response towards Ro60 in pSS and SLE [269]. There is evidence of intramolecular epitope spreading during the course of SLE [229,240].

La contains conformational and linear epitopes distributed over the whole protein, and the most prominent epitope appears to be situated in the RRM being recognized by 60% of La-reactive sera [270,271] (Fig. 10).

3.4.10.2. Role in diseases.

Neonatal lupus is almost always associated with maternal autoAbs to Ro60, Ro52 and La [272]. Neonatal lupus is characterized by transient lupus dermatitis, haematological and hepatic abnormalities and/or isolated congenital heart block (CHB). When mothers have connective tissue diseases with anti-Ro/SS-A antibodies, incidence of CHB is 1–2% in live births [273,274]. Tissue injury in CHB is thought to be mediated by the binding of maternal anti-Ro/La autoAbs to apoptotic cells in the developing fetal heart [275]. This may be particularly caused by Abs to Ro52, which may play a direct pathogenic role in congenital heart block by cross-reaction to the human serotoninergic 5-HT-4 receptor [6,276,277].

Anti-Ro/SS-A along with anti-La/SS-B autoAbs have also been detected in skin biopsies from patients with SCLE as well as in the infiltrated salivary glands of patients with pSS. Recent studies have shown that cultured epithelial cells from patients with pSS constitutively secreted exosomes that contained Ro/SS-A and La/SS-B, thus providing a source of intracellular autoantigens to the immune system [278].

Table 10
Autoantibodies to Ro60 and La.

	Anti-Ro60	Anti-La
Sjögren's syndrome	60–80%	40–60%
Systemic lupus erythematosus	40–60%	15–20%
Subacute cutaneous lupus erythematosus	70–100%	50%
Neonatal lupus	85%	60%
Rheumatoid arthritis		5–10%

3.4.11. Autoreactive T cells

In T-cell epitope mapping studies using HLA-transgenic mice, the majority of the dominant T-cell epitopes were located in the amino (aa 61e185) and carboxy (aa 381e525) terminal region of the Ro60 molecules [279] (Fig. 10). However, there are no reports about autoantigenic T cells in human disease so far.

3.4.12. Therapy

None established yet.

3.5. The ribosome

Ribosomes are the cell's centers of protein biosynthesis, translating the genetic code into protein sequence. Eukaryotic ribosomes contain a large (60S) and a small (40S) subunit, each of them composed of large ribosomal RNA (rRNA) molecules (18S rRNA and 28S rRNA, respectively) and approximately 80 proteins. Among these proteins only three (the acidic ribosomal phosphoproteins P0, P1, P2) are targeted by autoantibodies of patients with SLE. Forty years after their initial discovery of these autoantibodies, their potential correlation with disease manifestations of SLE is still controversial.

3.5.1. Size of autoantigens

P0: 317 aa (34.3 kD); P1: 114 aa (11.5 kD); P2: 115 aa (11.7 kD).

3.5.2. Structure

The acidic ribosomal proteins are organized into three distinct functional domains: an N-terminal domain that is responsible for oligomerization and ribosome association, a C-terminal domain implicated in interactions with translation factors, and a hinge region that allows a flexible relative orientation of the latter two portions [280] (Fig. 11). P0, P1 and P2 are organized in a pentameric complex with one copy of P0 and two heterodimers of P1 and P2.

3.5.3. Related proteins

Sequence comparisons show a large diversity in the stalk proteins across the evolutionary kingdoms, but a conserved functional domain organization and conserved designs of their genetic units are discernible. A 17-aa sequence in the carboxy-terminus that is responsible for interaction with elongation factors during protein synthesis shares almost complete amino acid-identity between human P0, P1 and P2 and their rat and yeast homologues [281].

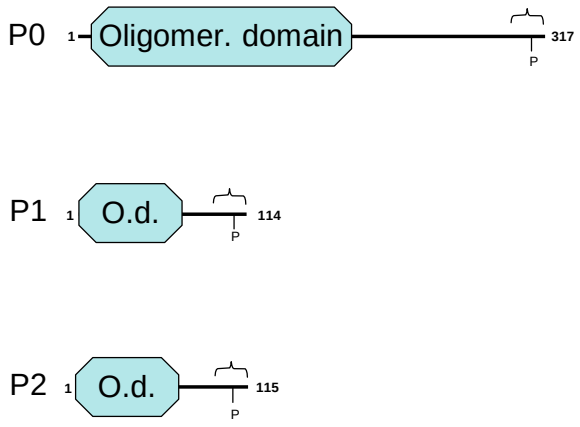

Fig. 11. Domain organization and epitopes of the acidic ribosomal proteins P0, P1, P2. A dominant linear B-cell epitope (depicted by a black parenthesis) is located within the C-terminal 22 aa region. This epitope is shared by all P proteins and appears to be associated with central nervous system-involvement. The epitope core contains a phosphorylated serine residue (P) that is dephosphorylated in a caspase-dependent process during Fas-L-induced apoptosis. Od, oligomerization domain; P, phosphorylation site.

3.5.4. Expression

P0, P1 and P2 are expressed in all cells. P1 and P2 mRNAs constitute roughly 0.01% of the total mRNA in HeLa cells, while P0 is several fold more abundant [281].

3.5.5. Location

In contrast to most other ribosomal proteins that are transported to the nucleus after their synthesis in the cytoplasm, incorporation of P1 and P2 into the ribosome takes place at a very late stage already in the cytoplasm while P0 is incorporated into the ribosome in the nucleus.

3.5.6. Biological function

The P0-$(P1\text{-}P2)_2$ pentamer forms the ribosomal stalk of the 60S (large) subunit, a protuberance where the elongation step of protein synthesis takes place. P0, P1 and P2 do not directly interact with ribosomal RNA but bind to the rRNAs via other proteins.

3.5.7. Knockout

The viability of eukaryotic cells not expressing P0, P1, and P2 does not seem to be affected, although cell growth is impaired [282].

3.5.8. Modifications

The eukaryotic P0, P1 and P2 proteins are phosphorylated at serine residues (Fig. 11). Phosphorylation of the acidic ribosomal proteins is crucial for the function of the ribosomal stalk by influencing the interaction with translation elongation factors as well as the interactions between P0, P1 and P2. Ribosomal proteins have been detected in ribosomal blebs of apoptotic cells and are dephosphorylated during apoptosis [283].

3.5.9. Autoantibodies

AutoAbs to the ribosomal P proteins (anti-rib P) show a high specificity for SLE, and have been only occasionally described in other autoimmune diseases. The prevalence in SLE is 6e46% and seems to be influenced by race, age and MHC class II haplotype of the patients (reviewed in [284]). Variability of prevalence figures may also rely to regional practice differences and selection of patients [285] (Table 11).

3.5.9.1. Epitopes. The conserved amino acid sequence at the carboxy-terminus constitutes the specific target of most anti-rib-P Abs. Thus, the majority of sera recognize all the three P proteins [286].

3.5.9.2. Pathogenic relevance. Despite thorough investigations the correlation between elevated titers of anti-rib-P Abs and SLE disease manifestations is still controversial. Most of clinical studies report an association with neuropsychiatric symptoms, particularly psychosis and depression. Furthermore there appear to be associations with nephritis [287], chronic hepatitis, and skin involvement in SLE. Binding of anti-rib-P Abs seems to block protein synthesis in vitro and in vivo, suggesting a possible pathogenic mechanism for these autoAbs.

3.5.10. Autoreactive T cells

Not known.

3.5.11. Therapy

There is no therapy specifically interacting with autoimmune recognition of ribosomal proteins yet.

Table 11
Autoantibodies to ribosomal antigens.

Systemic lupus erythematosus	6e46%
Other autoimmune diseases	<5%

3.6. Nucleolin

Also designed C23, nucleolin is a major nucleolar RNA-binding phosphoprotein. It possesses pleiotropic functions in several aspects of ribosome biogenesis and maturation as well as in the direct or indirect regulation of transcription, cell proliferation and growth, embryogenesis and nucleologenesis. Nucleolin acts as a shuttle between the nucleolus and the cytoplasm, thereby possibly targeting several ribosomal proteins to the nucleolus. Autoantibodies to nucleolin have been described in murine models of lupus and in patients with systemic lupus erythematosus and systemic sclerosis.

3.6.1. Size of autoantigen

Nucleolin is a phosphoprotein of 707 aa residues. Due to the presence of a stretch of negatively charged residues in its N-terminal region and of phosphorylated residues, the apparent molecular size of nucleolin in SDS-PAGE is 105 kD while its actual mass calculated from the cDNA sequence is only 77 kD. Shorter nucleolin forms of various molecular masses are generated by a covalently attached protease and by auto-cleaving and can be observed predominantly in nondividing cells [288].

3.6.2. Structure

Nucleolin is encoded as a single copy gene of w9 kb with 14 exons and has a modular organization. It consists of an acidic N-terminal domain that controls rDNA transcription, a central globular domain exhibiting alternating hydrophobic and hydrophilic stretches that controls pre-RNA processing, and a C-terminal domain that controls nucleolar localization [288,289] (Fig. 12). The N-terminal domain, which contains HMG protein-like regions formed by lengthy acidic stretches and a-helix domains, is variable and less conserved in different species. The central domain of the protein contains four RBD that are present in many RNA-binding proteins. The presence of a bipartite nuclear localization signal located between the N-terminal and the RNA-binding (central) domain has been characterized and is necessary for nucleolin to enter the nucleus [290]. The C-terminal domain is unusually rich in glycine, arginine and phenylalanine residues (RGG domain). The RGG domain of nucleolin binds RNA non-specifically and with low affinity and is also involved in proteineprotein interactions [291,292].

3.6.3. Related proteins

Orthologs and nucleolin-like proteins have been described in mammals, chicken, Xenopus laevis, plants, bacteria and yeast [293].

3.6.4. Expression

Nucleolin is barely detectable in resting cells but represents 5% of nucleolar protein in actively dividing cells [289].

3.6.5. Location

Nucleolin is present in abundance at the dense fibrillar and granular regions of the nucleolus. It has also functions outside of the nucleus and shuttles between the nucleolus and the cytoplasm. Furthermore, although nucleolin does not contain any transmembrane domain allowing its association at the plasma membrane, it is also present in its phosphorylated form at the membrane surface of various cell types. Membrane nucleolin is closely associated to intracellular actin filaments, probably via a still unknown transmembrane partner. Nucleolin is overexpressed in numerous cancers and in actively proliferating cells. Nucleolin present in the cytoplasm is translocated to the membrane surface by an active, non-conventional mechanism [294].

3.6.6. Biological functions

Nucleolin possesses pleiotropic functions in several aspects of ribosome biogenesis and maturation, as well as in the direct or indirect regulation of transcription, cell proliferation and growth, embryogenesis and nucleologenesis [288,293,295]. It acts as a shuttle between the nucleolus and the cytoplasm, thereby possibly targeting several ribosomal proteins to the nucleolus. Nucleolin binds to DNA and single-stranded nucleotidic sequences. In the nucleus, many proteins also bind to nucleolin. For example, it associates with casein kinase II [296], topoisomerase-I [297], the B23/nucleophosmin protein [298], and with several ribosomal proteins [291]. It also binds to the hY1 and hY3 RNPs, and associates with Ro RNP particles subsets containing one of these two hY RNAs. It has been hypothesized that nucleolin is involved in the biogenesis and/or trafficking of hY1 and hY3 RNPs to the cytoplasm [299].

At the cell surface, nucleolin behaves as a receptor of moderate affinity for a variety of ligands including growth factors such as midkine and heparin affin regulatory peptide, also known as pleiotrophin [300], lactoferrin [301], apoB- and apoE-containing lipoprotein [302], laminin [303], and different viruses such as Coxsackie B virus and HIV-1 [304]. These ligands are transported intracellularly using nucleolin as a carrier. This mechanism is active and probably requires lipid rafts. Using deletion mutants of nucleolin, it has been demonstrated that the RGG motif repeated nine times in the C-terminus of the protein, encompasses the binding site for these ligands. Thus nucleolin can act as a "shuttle service" both from the nucleolus to the plasma membrane and from the external membrane to the cytoplasm.

3.6.7. Knockout

No human mutations in nucleolin have been reported, nor have nucleolin knockout mice been created. Studies in yeast showed that when the gene encoding the homolog Nsr1 is disrupted, the yeast survive but with a severe growth defect [305].

3.6.8. Modifications

Nucleolin is subjected to several types of post-translational modifications that interfere with its functions [288]. There is an interplay of signals involving phophorylation/dephosphorylation of nucleolin and its proteolytic fragments leading to the regulation of its many functions during the different phases of the cell cycle (chromatin organization, rRNA packaging, rDNA transcription and ribosome assembly). Serine phosphorylation of nucleolin by casein kinase II occurs during interphase whereas phophorylation of threonine residues by cdc2 kinase occurs during mitosis [306]. Nucleolin is also phosphorylated by cAMP-dependent protein kinase, protein kinase C-X, and ecto-protein kinase. Another interesting structural feature of the nucleolin C-terminal domain is the

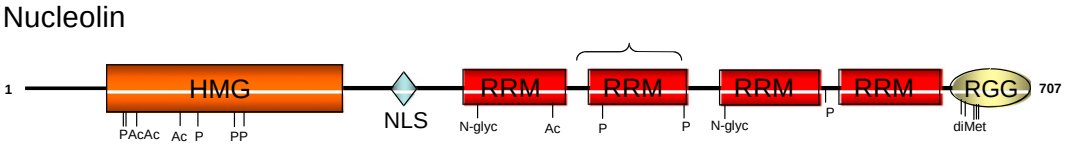

Fig. 12. Domain organization and epitopes of nucleolin. The only known B-cell epitope recognized by a patient with SLE (aa 387e461) is depicted by a black parenthesis. Ac, acetylation site; diMet, dimethylation site; HMG, high-mobility group protein-like region; N-glyc, N-glycosylation site; NLS, nuclear localization signal; P, phosphorylation site; RGG, Arginine and glycine-rich region; RRM, conserved RNA-recognition motif.

Table 12
Autoantibodies to nucleolin.

	Number of positives
Childhood systemic lupus erythematosus	15/15 (lgM and/or IgG)
Adult systemic lupus erythematosus	27/42 (IgM) 7/42 (IgG)
Acute hepatitis	16/20 (lgM and/or IgG)
Infectious mononucleosis	4/20 (IgM and/or IgG)

From [310].

presence of dimethylarginine residues, which are also found in the spliceosomal SmD1 protein [307]. Nucleolin also undergoes partial N- and O-glycosylations in the extranuclear cell compartment [308] (Fig. 12).

3.6.9. Autoantibodies

AutoAbs to nucleolin have been shown to occur in SLE, SSc and scleroderma-like chronic graft-vs-host disease, and primary biliary cirrhosis [309e311] (Table 12). They have also been detected in sera from MRL-lpr mice [312].

3.6.9.1. Epitopes. Little information is available. Using deletion fragments of nucleolin, an epitope has been localized in residues 387e461 [313]. It is not known whether as in other RNA-binding proteins, the nucleolin RBD represents an immunodominant region preferentially targeted by autoAbs (and autoreactive T cells). A systematic mapping of nucleolin (linear) epitopes has not yet been done.

3.6.9.2. Role in diseases. Redistribution of nucleolin occurs within the cell in response to a number of stimuli, including heat shock, mitosis, T cell activation, treatment with a cyclin-dependent kinase inhibitor, and viral infection. Such nucleolin changes are associated to malignity, inflammation, and proliferation, but are not known as a causal agent for any disease.

3.6.10. Autoreactive T cells

Unknown.

3.6.11. Therapy

Autoimmunity to nucleolin has not been selected for therapeutic intervention so far. However, nucleolin ligands might have promising pharmacological applications, notably in anti-cancer strategies and in the control of neurological diseases.

3.7. Aminoacyl-tRNA synthetases

The aminoacyl-tRNA synthetases (aaRS) are a group of cytoplasmic proteins that catalyze the charging of tRNAs with their respective amino acids. Autoantibodies to aaRSs are specific for polymyositis/dermatomyositis, rendering them a high diagnostic value. The most important antibodies (termed anti-Jo1 antibodies) are directed towards histidyl-tRNA synthetase. They are detectable in about 30e40% of patients with myositis and are associated with interstitial lung fibrosis, giving them also high prognostic value. Antibodies to alanyl-, glycyl-, and threonyl-tRNA synthetases are less prevalent, occurring in about 2e5% of myositis patients, while antibodies to other aminoacyl-tRNA synthetases are extremely rare.

3.7.1. Size of the major autoantigens

Histidyl-tRNA synthetase (HARS): 509 aa (57.4 kD); HARS2: 506 aa (56.9 kD); Threonyl-tRNA synthetase (TARS): 723 aa (83.4 kD). TARS2: 718 aa (81 kD); Alanyl-tRNA synthetase (AlARS): 968 aa (106.81 kD). AARS2: 985 aa (107.34 kD); Glycyl-tRNA synthetase (GARS): 739 aa (83.14 kD).

3.7.2. Structure

AaRSs are multidomain proteins. Typically, an aaRS consists of a catalytic domain and an anticodon-binding domain (which mostly interacts with the anticodon region of the tRNA and ensures binding of the correct tRNA to the protein). In addition, some aaRSs have additional RNA-binding domains and editing domains that cleave incorrectly paired aminoacyl-tRNA molecules (Fig. 13). From the primary structures, 2 distinct classes of synthetases have been recognized, with similarity of certain structural features, amino acid attachment sites, and other properties among members of a class. Class I aaRSs have two highly conserved sequence motifs, aminoacylate at the 2^{0}-OH of an adenosine nucleotide, and are usually monomeric or dimeric. Class II aaRS, among them all autoantigenic synthetases, have three highly conserved sequence motifs, aminoacylate at the 3^{0}-OH of the same adenosine, and are usually dimeric or tetrameric. They have a unique fold made up of antiparallel beta-strands.

3.7.3. Related proteins and evolutionary conservation

HARS2, a mitochondrial version of HARS, shares 72% amino acid-identity with HARS [314]. Similarly, there are mitochondrial forms of other aaRSs [315]. Patients' Abs appear to react with the cytoplasmic forms only. There are several HARS transcripts that differ only in the lengths of their 5-prime UTRs. The catalytic domains of all the aaRSs of a given class are found to be highly homologous, while class I and class II aaRSs are unrelated to one another. Pufferfish and human HARS share 84% aa identity, demonstrating a high degree of conservation across species [314].

3.7.4. Associated nucleic acids

AatRNAs associate exclusively with their specific tRNAs.

3.7.5. Expression

AaRSs are indispensable for translation and therefore necessarily present in all nucleated cells. HARS has shown to be particularly highly expressed in heart, brain, liver, and kidneys.

3.7.6. Location

Largely cytoplasmic.

3.7.7. Biological function

AaRSs catalyze the ATP-dependent esterification of an amino acid to its cognate tRNA. Some synthetases also mediate a proofreading reaction to ensure high fidelity of tRNA charging; if the tRNA is found to be improperly charged, the aminoacyl-tRNA bond is hydrolyzed.

3.7.8. Knockout

AaRSs are essential in all cell types. The mouse sticky mutation, that causes cerebellar Purkinje cell loss and ataxia, is a missense mutation in the editing domain of AARS that compromises its proofreading activity, thus leading to accumulation of misfolded proteins, cell death and neurodegeneration [316].

3.7.9. Autoantibodies

PM/DM patients exhibit specific autoAbs to at least six aaRSs, each reacting with a single enzyme, of which the most common are anti-Jo-1 autoABs which are directed to HARS (Table 13). Although anti-aaRS Abs are normally directed against the protein component, they can occasionally also target the tRNAs itself [317]. AutoAbs to different aaRSs usually do not occur together in the same myositis serum, whereas they can occur in combination with other autoAbs. Per example, an association between anti-Ro52 autoAbs and anti-Jo-1 has been reported [318]. It has been suggested that autoAbs in myositis develop due to apoptotic granzyme B-mediated cleavage of antigens that leads to formation of new epitopes [319].

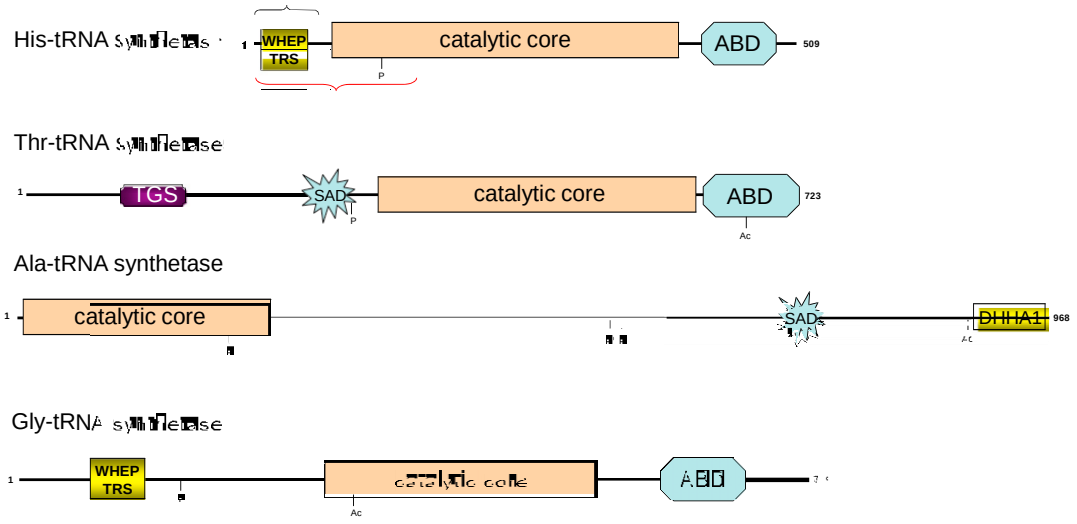

Fig. 13. Domain organization and epitopes of the aminoacyl-tRNA synthetases. Both B-cell epitopes (black parenthesis) and T-cell epitopes (red parenthesis) of HARS appear to be confined to the amino terminal part of the protein. ABD, anticodon-binding domain; Ac, acetylation site; DHHA1; DHH-associated domain; P, phosphorylation site; SAD, second additional domain; TGS, ThrRS, GTPase, and SpoT domain of unknown function; WHEP-TRS, RNA-binding domain.

Anti-Jo-1 autoAbs are associated with DRB1 *0301, whereas anti-aaRS Abs other than anti-Jo-1 are associated with DR52 [320].

3.7.9.1. Epitopes. Most or all of the autoreactive activity towards HARS is directed towards a structural epitope comprised in the N-terminal 60 aa. Little is known about B-cell epitopes on other aaRSs.

3.7.9.2. Role in diseases. Myositis is seen in over 90% of patients with anti-Jo-1, anti-EJ, or anti-PL-7 Abs. Anti-aaRS Ab positive patients have higher frequencies of interstitial lung disease, arthritis, Raynaud's phenomenon, and hyperkeratosis on the edges of the fingers, and generally show a poor prognosis [321]. Therefore this more severe form of autoimmune myositis is also known as the anti-synthetase syndrome. Although anti-Jo1 Abs may fluctuate with disease activity and anti-Jo-1 titer, there is no clear evidence of a direct pathogenic involvement of any of these autoAbs.

3.7.10. Autoreactive T cells

PM is characterized by oligoclonal T cell infiltrates causing inflammation and mediating muscle weakness. There is good evidence that formation of anti-Jo-1 autoAbs is antigen-driven and therefore dependent on $CD4^b$ T cells that may also direct cytolytic $CD8^b$ T cells involved in myocyte destruction. T cell proliferative responses are MHC class II-dependent and directed against a unique N-terminal fragment of HARS [322] (Fig. 13).

3.7.11. Therapy

Tolerization against aaRS has not been tried so far.

4. The role of nucleic acids in autoimmune diseases

Since nucleic acid-associated antigens are conspiciously often targeted in systemic autoimmune diseases, TLR-activation by nucleic acids has been postulated to play a role in initiation of autoimmune reactions. TLR stimulation leads to the activation of transcription factors such as NF-kB and IRF3, resulting in the production of proinflammatory cytokines, particularly type I IFNs, IL-6, and TNFa. APCs activated by TLR ligation induce Th1-differentiation, which has been shown to be a contributing factor to disease pathogenesis in models of diabetes and Crohn's disease [323,324], and a role for TLRs in the maturation of DCs has been documented in models of MS, RA, and SLE [181,325,326]. Moreover, the suppressive function of Tregs can be inhibited by TLR stimulation [327,328].

These observations and data obtained with other nucleic acid-sensing systems have led to the concept that nucleic acids and nucleic acid-sensing receptors are crucially involved in the generation of pathogenic autoimmune reactions, at least in systemic autoimmune diseases. In this concept, known as the Toll hypothesis of autoimmunity, it is hypothesized that the nucleic acid components of macromolecular complexes may act as endogenous triggers to drive APC activation while the protein component is subsequently processed by the activated APCs and presented to T cells, which will become activated and promote inflammatory reactions and autoAb production, leading eventually to autoimmune diseases such as SLE, MCTD, SSc, PM/DM, SS, or RA [154,329].

Table 13
Autoantibodies to aminoacyl-tRNA synthetases in PM/DM.

Histidyl-tRNA synthetase (anti-Jo-1)	20e30%
Threonyl-tRNA synthetase (anti-PL-7)	2e5%
Alanyl-tRNA synthetase (anti-PL-12)	2e5%
Glycyl-tRNA synthetase (anti-EJ)	2e5%
Other aminoacyl-tRNA synthetases	below 2%

5. Novel roads for immunotherapy of autoimmune diseases

Current therapies for autoimmune diseases still mainly suppress inflammation and only indirectly affect antigen-specific immune activities. However, therapeutically targeting relevant autoantigens has the potential to deliver maximal efficacy with minimal adverse effects. Despite promising results in mouse models, antigen-specific immunotherapy has not yet been very successful. A few on-going clinical trials only hold promise and might represent future roads for immunotherapy in autoimmune diseases [330].

5.1. Strategies targeting intracellular components

Targeting intracellular processes, such as signalling, apoptosis or the cell cycle may represent novel roads for immunotherapy in autoimmune diseases. A number of intracellular components are currently tested with small molecules and peptide tools for their

therapeutic potential [331]. Examples of promising compounds are rapamycin or tracolimus that bind to the specific cytosolic binding-protein FKBP12, and celecoxib or celebrex that inhibit cyclo-oxygenase-2.

Toll-like receptors such as TLR7 and TLR9 also represent potentially important targets for immunoregulating autoimmune diseases. There is increasing evidence that chronically activated pDCs and the IFNa they produce in response to stimulation of TLR7 and TLR9 by nucleic acids play an important role in the pathogenesis of autoimmune diseases such as SLE, pSS, or RA. Patients with SLE have highly decreased numbers of pDCs in the blood, probably due to cell migration into peripheral lymphoid tissues and sites of inflammation (such as cutaneous lupus lesions) after activation [332,333]. As the non-TLR pathways involved in nucleic acid recognition such as RIG/MDA5 appear to be weak or absent in human pDCs [334] and since the role of the cytosolic DNA sensor DAI has been questioned in human cells [335], blocking TLR7 and TLR9 could be sufficient to block the entire response of pDCs, including type I IFN-production.

The antimalarial drug chloroquine has been shown to block the immune stimulatory effects of CpG DNA, U1 snRNP and their immune complexes at low concentrations [181,182,336] by inhibiting TLRs 7/8/9. Chloroquine-related TLR antagonists may thus be used to prevent apoptotic debris from stimulating and sustaining unwanted and dangerous autoimmune reactions. The development of other more specific TLR antagonists could make major contributions to the treatment of autoimmune diseases. Of note, TLR blockage bears the potential danger of increased secondary infections [337]. However, MyD88-deficient mice have remarkably mild phenotypes, suggesting that TLRs are essential for the response to only a few specific pathogens and are otherwise redundant [338]. Several possible methods designed to inhibit TLR signalling more specifically than chloroquine are currently under investigation by different groups and notably include the development of so-called immunoregulatory DNA sequences (IRS), that are atypical, non-stimulatory DNA sequences that inhibit TLR stimulation (also known as suppressive or S-class ODNs). Synthetic ODNs, which act as antagonists for TLR7 or TLR9 [181] and bimodal ODNs (blocking both TLR7 and TLR9) have been tested. IRS most likely act as competitive receptor-antagonists. TLR9 appears to exist as a preformed heterodimer in endosomes. Binding of a stimulatory CpG sequence causes a conformational change that brings the cytoplasmic domains closer together resulting in signal transduction [339].

TLR9 inhibitors [340,341] have already shown therapeutic effects in models of sepsis, SLE, and arthritis [342,343] and block virus-induced IFNa-production [181] and IFNa-production induced by immune RNP containing complexes [344]. Bimodal IRS were found to reduce levels of autoAbs in (NZB × NZW)F1 mice, a spontaneous mouse model dependent on IFNa [345,346], reduced proteinuria and end-organ damage and increased survival [347]. No change however was observed in the lymphocytic infiltration in the kidney. Interestingly, TLR9 activation has been reported to be beneficial in MRL-lpr lupus mice [348,349]. It should be noted that in this model the disease is strongly accelerated by a defect in the fas gene whereas lupus patients with mutations in fas are very scare [350]. Furthermore, rather than IFNa, IFNg appears to play a major pathogenic role in this lupus mouse model [351e353]. Thus, the use of dual inhibitors that would block both TLR7 and TLR9 could be considered to be a promising strategy for patients with SLE.

5.2. Strategies targeting cell surface-expressed molecules

Over the last decade, intense research has led to the development of cell surface-based therapeutics, which have been recently introduced to the market or are currently under clinical evaluation in patients. These are for example monoclonal antibodies (mAbs) targeting B cells, such as rituxan (rituximab) or ocrelizumab (mAbs to CD20 antigen on B cells; both in phase III by Genentec), LymphoStat-B (belimumab/Benlysta; phase III by Human Genome Sciences and GlaxoSmithKline) that targets B-lymphocyte stimulator (BLyS), and epratuzumab, a humanized Ab that targets the CD22 receptor on B cells (phase IIb by UCB Pharma). Other cell surface-expressed molecules that can be targeted are CD3, CD25, CD52 (at the surface of mature lymphocytes), CD28 interacting with CD80/86, CD40 and CD40 ligand, sphingosine-1-phosphatase receptor, and certain integrins, for example (for recent reviews see [331,354,355]). Atacicept, a TACI-Ig fusion protein currently evaluated by Zymogenetics/Merck Serono in placebo-controlled phase II/III clinical trials for lupus, targets BLyS and APRIL, two members of the TNF family which promote B cell-survival. In an earlier phase Ib trial, the drug was shown to be safe and well-tolerated with no serious adverse effects and there was a positive trend in SELENA-SLEDAI scores, and complement levels in treated patients [356].

5.3. Strategies targeting soluble effectors of the inflammatory response in autoimmune diseases

Cytokines, such as TNFa, IL-1 and IL-6 are important targets of efficient biological therapies [357,358]. Although very promising results have been shown with anti-TNFa medications, the blockade of such an important immune pathway requires a very careful and continuous safety monitoring to avoid detrimental side effects. Even though non-specific, this strategy has already considerably modified the management of patients and ameliorated their quality of life.

Among the novel roads for treating patients with anti-cytokine tools are strategies of active vaccination based on whole cytokines or peptides that mimic natural cytokines. Peptides derived from IL-1b and TNFa, for example, were found to inhibit disease in animal models of RA when administrated in the presence of adjuvant [359,360]. Here again, however, a careful follow-up is required to examine long-term effects and possible risks.

Other soluble molecules such as hormones and elements of the complement pathway are also currently extensively studied. Although theoretically pertinent, these targets are still at an experimental stage of study.

5.4. Antigen-specific immunotherapy

Autoreactive T cells represent central targets for antigen-specific strategies to treat autoimmune diseases. Numerous reports show the therapeutic potential of antigen-specific T cell-mediated tolerance. Thus, histone peptides encompassing epitopes recognized by $CD4^b$ T cells from lupus mice (regions 16e39 or 71e93(94) of H4, 111e130 of H3, and 22e42 of $H1^0$, respectively) were shown to restore tolerance in these mice [156,157,301e303]. Most of these peptides are also recognized by human T cells [37]. Other examples are peptides derived from the laminin a-chain successfully tested in MRL-lpr lupus mice [361], and from the 60-kD heat-shock protein (hsp60) evaluated in a Sjögren's syndrome nonobese diabetic (NOD) mouse model [362]. The hsp60 peptide 336e351 was also tested for preventing relapses of uveitis in Behcet's disease, a multisystemic disease affecting mucocutaneous, ocular, central nervous system, joint, and vascular tissues. Oral administration of this peptide was found to be safe and in responder patients several signs of tolerization were noted [363].

Several peptides corresponding to T-cell epitopes identified in the insulin B chain were also tested for their ability to protect NOD mice from diabetes [364e367]. To our knowledge, however, administration of proinsulin or insulin peptides (or peptide analogues) has not been attempted in the context of type 1 diabetes prevention trials or

in an attempt to halt autoimmunity in new onset patients (for a recent review see [368]).

Peptide analogues of myelin basic protein (MBP), myelin oligodendrocyte glycoprotein and myelin proteolipid protein have been extensively studied to prevent experimental autoimmune encephalomyelitis (EAE), an animal model of multiple sclerosis (MS). Peptide analogues and notably altered peptide ligands that have the capacity to deviate the signal transduction events following their interaction with the T cell-receptor were designed, and some of them were found to partially restore the normal immune response in mouse models (for a recent review see [330]). Promising results were obtained with ATX-MS-1467, a preparation made of a mixture of four soluble MBP peptides tested in an open phase I/IIa clinical trial involving patients with MS (Apitope Technology Ltd, Bristol, UK). A double-blind placebo-controlled phase II study has been announced by Merck Serono (Darmstadt, Germany) in 2009.

Another interesting compound is Cop-1 (Copolymer-1, glatiramer acetate, Copaxone), a synthetic copolymer composed of 40–100 residues of the four amino acids glutamate, lysine, alanine, and tyrosine, associated in a defined molar ratio [369]. This complex peptide-construct immunologically mimics MBP and suppresses EAE in several animal species. Cop-1 is also efficient in humans and was shown to slow down the progression of disability and to reduce the relapse rate in patients with exacerbating-remitting MS. It was approved by the Food and Drug Administration in 1995, and is used today by tens of thousands of patients [370]. The mechanism of action by which Cop-1 is beneficial in central nervous system autoimmune disease is thought to be mediated through a preferential Th2 deviation of myelin-specific T cells [371]. Cop-1 also prevents graft-vs-host disease and transplant rejection and may also be effective in models of other autoimmune diseases such as experimental uveoretinitis and inflammatory bowel disease.

So far, only few data are available concerning peptide-based treatment of patients with systemic autoimmune diseases. Recently, a peptide derived from the CDR-1 of an anti-DNA Ab was shown to ameliorate disease lupus mouse models and also in patients with SLE [41,42]. A last promising example of antigen-specific immunotherapy based on peptides recognized by autoreactive T cells involves a segment of the spliceosomal U1-70K snRNP autoantigen. The peptide P140 that has been described in detail above has been shown to improve proteinurea and anti-DNA Ab production in MRL-lpr mice when given intravenously and also holds promise for a successful treatment of human SLE [163,186,193].

6. Concluding remarks

In the past years, a tremendous amount of research concerning the molecular and cellular mechanisms involved in the onset and the pathogenesis of autoimmune diseases has been performed. Data that emerge from these numerous studies reveal a variety of pathways that were sometimes ignored until now or poorly studied, and that are central in the break of tolerance and the subsequent development of autoimmunity and inflammation. Some of these regulatory pathways belong to innate immunity that was not properly integrated previously in the classical schemes of autoimmunity. This opens new possibilities for innovative strategies that may be highly effective. A number of limitations that remain a challenge in our quest of efficient therapeutics concern the fine specificity of tools, their stability and bioavailability, their potential to target the exact molecule at the desired site selected for their action, and their non-toxicity. Dozens of therapies are in various stages of clinical development for diseases such as SLE, RA, MS, and diabetes. Efforts are still required but certainly, better therapies will emerge soon, which will permit to decrease the frequency of flares and relapses in autoimmune patients.

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