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Primary Biliary Cirrhosis and the Nuclear Pore Complex

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Abstract

Experimental models of autoimmune diseases have led to the conclusion that an immune response to nuclear antigens is a sentinel marker for loss of tolerance and potential tissue damage. Various proteins are targets of antinuclear antibodies in a variety of autoimmune diseases, ranging from systemic rheumatologic disorders to diseases affecting specific organs such as the liver. Autoantibodies against specific nuclear constituents have also been used as probes to understand the structure and the function of the targeted components and their relevance to disease pathogenesis. Approximately a quarter of patients with primary biliary cirrhosis (PBC) have antibodies targeting proteins of the nuclear pore complex (NPC), a multi-protein structure that mediates molecular transport across the nuclear envelope. Autoantibodies against the integral membrane glycoprotein gp210 and nucleoporin p62 appear to be highly specific for PBC, an autoimmune disease characterized by progressive destruction of intrahepatic biliary epithelial cells. This review discusses the diagnostic and clinical relevance of anti-NPC antibodies in PBC and the possibility that this autoimmune response may arise as a result of molecular mimicry.

Keywords

autoantibody; autoantigen; autoimmunity; nuclear envelope; nuclear pore complex; nucleoporin; primary biliary cirrhosis

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INTRODUCTION

Loss of immunological tolerance to the nucleus is among the best-studied topics in autoimmunity, largely due to the fact that anti-nuclear antibodies (ANA) are frequently present in a variety of autoimmune diseases [1–11]. However, the mechanisms responsible for the induction of immune responses against distinct nuclear antigens and their relevance to specific diseases remain elusive. ANA can be predictive of the future development of autoimmune disease and present years or possibly even decades before the onset of clinically evident disease [9]. To complicate the matter, ANA can be found at relatively low titers in up to 5% of "healthy" individuals, with the prevalence increasing with age. In some cases, ANA may be irrelevant to pathogenesis, possibly conferring protection from development of disease [12–14].

ANA are generally detected in clinical laboratories by indirect immunofluorescence microscopy (IIF) using as a substrate HEp-2 cells, a human laryngeal carcinoma line. HEp-2 cells are usually selected because they have large nuclei and cells in the preparations can be present in various stages of mitosis, allowing for discrimination of distinct staining patterns [15–18]. Nuclear fluorescence indicates not only the presence of ANA but also the localization of reactive antigens within the nucleus, as characteristic staining patterns are frequently correlated with specific diseases [15, 18, 19].

ANA in serum samples from patients with PBC often produce a rim-like pattern when examined by IIF, suggesting that the targets of the autoantibodies are components of the nuclear envelope (NE) [20–24]. A smooth rim-like fluorescence pattern suggests antibody recognition of an antigen of the nuclear lamina or inner nuclear membrane whereas a punctate pattern suggests that the recognized antigen is a component of the nuclear pore complex (NPC) [15, 19]. Autoantibodies specific for constituents of the NE antibodies have also been described in other autoimmune diseases sometimes associated with PBC, such as Sjögren's syndrome [25]. Additionally, such autoantibodies are occasionally present in systemic lupus erythematosus (SLE) and mixed connective tissue disorders [26, 27]. These findings have prompted a series of investigations to define the autoantigens of the NE in PBC and to attempt to dissect their role in pathogenesis.

The NE

The NE is a highly organized membranous structure [28] divided into the nuclear membranes (outer, inner and pore domains), NPCs and the nuclear lamina. The outer nuclear membrane is directly continuous with the rough endoplasmic reticulum and the perinuclear space separates the inner from the outer membrane. The inner and pore membranes contain unique sets of intrinsic and extrinsic proteins [28, 29]. The lamins, intermediate filament proteins that form the nuclear lamina, are extrinsic proteins of the inner nuclear membrane. Some transmembrane proteins freely diffuse between the inner, outer and pore membrane domains of the NE without concentrating in any of them. NPCs are located at sites at where the inner and outer membranes fuse to form the pore membranes. Most of the protein building blocks of the NPC are called nucleoporins, some of which are transmembrane proteins of the pore membrane and most of which are non-membrane proteins of the complex.

The NPC

The number of NPCs varies among different cell types of different species. Among mammals, there are approximately 3,000 to 5,000 NPCs per nucleus. During interphase, the passage of molecules from and to the nucleus occurs strictly via the NPC. It is ~100–120 nm in diameter with a central transport channel measuring ~40 nm in diameter. The aqueous

central transport channel allows for the exchange of macromolecules including RNA, proteins and ribonucleoproteins across the nuclear envelope, a process assisted by soluble transport receptors [29, 30]. NPCs are also involved in chromatin organization, control of gene expression and replication-coupled DNA repair [29]. Electron microscopy and more recently X-ray crystallography have helped elucidate the structure and unique architecture of NPCs, in addition to providing insight as to how these macromolecular structures regulate the bidirectional exchange of macromolecules between the cytoplasm and nucleus [29, 31]. NPCs have a central doughnut-shaped central core with an eightfold rotational symmetry associated with cytoplasmic filaments and a nuclear basket, each composed of complexes of various nucleoporins (Figure 1) [29, 31]. This distinctive architecture gives to NPC the flexibility required for the dynamic macromolecular translocation [31–33].

NPCs were first described sixty years ago with the advent of electron microscopy; however, mechanistic details regarding their functions have only recently been elucidated [34–37]. The nucleoporins comprise a family of at least 30 different evolutionary conserved proteins [29, 32, 33, 38, 39]. Each nucleoporin exists in multiple copies, and approximately 500–1,000 protein molecules are present in the fully assembled NPC. A nucleoporin is generically denoted as Nup, followed by a number that refers to its molecular mass. However, a uniform nomenclature has not yet been agreed upon and the molecular masses of nucleoporins vary among species. Several authors have adopted a classification based on the approximate localization within the NPC. This classification has led to six categories including: (A) integral membrane proteins of the pore membrane domain of NE; (B) membrane-apposed coat nucleoporins; (C) adaptor nucleoporins; (D) channel nucleoporins; (E) nuclear basket nucleoporins; and, (F) cytoplasmic filament nucleoporins.

Antibodies against gp210 in PBC

In the late 1980s, Lozano et al [23] and Lassoued et al [21] demonstrated that serum samples from patients with PBC give a rim-like pattern when examined by IIF and that the antibodies recognized a NE protein with a molecular weight of approximately 200 kDa. A subsequent study by Courvalin et al identified this protein as gp210 [40]. Nickowitz et al [41] expressed a full-length recombinant gp210 and showed that all serum samples from patients with PBC reacting with the 200 kDa protein recognized the recombinant protein, thus confirming the identity of gp210 as the 200 kDa target in PBC.

Gp210 is a type I integral membrane protein that anchors NPCs to the pore membrane [42, 43]. It has a cytoplasmic C-terminal tail domain that faces the nuclear pore complex and an N-terminal domain located in the perinuclear space. The C-terminal tail of gp210 consists of 58 amino acids while the luminal domain contains 1808 amino acids (including the signal sequence) with several N-linked oligosaccharides. Gp210 likely participates in the events that take place when a new nuclear pore complex is formed, as well as the transmission of signals from the perinuclear cisterna to the nuclear pore [44]. There are data to suggest that gp210 is organized into the pore membrane as a large array of dimers that may form a luminal submembranous protein skeleton [44].

Anti-gp210 antibodies in PBC are immunoglobulin G isotype and primarily recognize a 15-amino acid linear stretch within the C-terminal domain of the protein [45]. A second subdominant epitope at the N-terminal end of the antigen has also been described but the significance of this finding remains elusive [46]. Most studies investigating the prevalence of anti-gp210 antibodies in patients with PBC have used purified extracts of NE proteins from various cell preparations or recombinant or synthetic polypeptides corresponding the C-terminal epitopic region. The majority of these studies reveal a ≥96% specificity of anti-gp210 antibodies for PBC [21, 23, 25, 41, 46–54]. Depending on the assay and the cohort,

the prevalence of anti-gp210 in PBC varies, ranging from 9.4%[41] to 41.2% [23]. Most studies report a prevalence of anti-gp210 antibodies in PBC within a 22–32% range [21, 46–48, 50–54]. A multicenter study including 1,175 patients with PBC from North America, Europe and Japan reported anti-gp210 antibody reactivity in 17.9% (210 cases) patients with PBC [55]. The same study demonstrated that anti-gp210 antibodies were slightly less prevalent in patients with a diagnosis of PBC but without antimitochondrial antibodies (AMA) detected by IIF (37/253, 14.6%) compared to those with detectable AMA (174/922, 18.9%). Similar findings have been obtained in another cohort of patients with PBC [56].

Antibodies against Nup62 in PBC

Nup62 was the first nucleoprotein to be identified and characterized [38]. It is a 60kD glycoprotein, which forms a subcomplex with nucleoporins p54, p58/p45 [57, 58]. Like one third of nucleoporins, p62 contains phenylalanine-glycine repeats. These repeats are part of larger motifs, which constitute binding sites for nuclear transport receptors and are involved in mRNA export [29, 59].

In contrast to antibodies against gp210, the significance of antibodies against nup62 in PBC remains less well established. Wesierska-Gadek et al [60] reported anti-nup62 antibodies in 32% of patients with PBC. In two studies, Miyachi and colleagues [51] reported the presence of anti-nup62 antibodies in 7 of 31 (22%) and 40 of 128 (31%) of patients with PBC [25]. Wesierska-Gadek et al [61] et al provided data suggesting that a highly-sensitive assay based on immunoprecipitation of recombinant antigen may more frequently detect reactivity to nup62 in PBC [62]. Some studies have also reported anti-nup62 reactivity in SLE and Sjögren's syndrome [25–27, 63]; however, whether these patients had concomitant PBC is not clear [51]. Kraemer et al studied a small cohort of patients with SLE and detected anti-nup62 antibodies in 6 out of 25 (24%) [27]; however, the control group only included two unaffected individuals, raising concerns about the specificity of their assay. Kreamer et al. also reported an association between anti-nup62 antibodies and renal damage or advanced neuropathy but this correlation has not bee repeated [27]. No sera from patients with SLE were included in the control groups tested by Miyachi et al [25, 51] and Wesierska-Gadek [60–62, 64], leaving unanswered the question as to the prevalence of anti-nup62 in this disease. The simultaneous presence of gp210 and nup62 autoantibodies appears to be a characteristic feature of PBC [51] with few exceptions [63].

A single study has shown that anti-nup62 antibodies are directed against an epitope within the amino terminal of the protein [26]. Preliminary results published by Meda et al [65] suggest that the region spanning amino acids 473 to 499 of nup62 is the major CD4+ T cell epitope. Some evidence suggests a lack of distinct linear B-cell epitopes on nup62 [65].

Clinical significance of anti-NPC antibodies in PBC

Early studies provided inconsistent results regarding the clinical significance of anti-gp210 antibodies in PBC. Lassoued and colleagues originally reported no difference between patients with and without detectable anti-gp210 antibodies in regards to biochemical indices of liver disease, clinical outcome or histological lesions [21]. Patients with anti-gp210 antibodies less frequently had PBC-related extrahepatic diseases, such as arthralgias and Raynaud's phenomenon, and had significantly lower titers of AMA [21]. A retrospective study of 159 patients with PBC also failed to demonstrate a correlation between the presence of anti-gp210 antibodies and prognosis, although this study reported a higher incidence of arthritis in patients [41]. A multi-center Japanese study did not reveal a relationship between the presence of anti-gp210 antibodies and histological staging in patients with PBC [25]. In contrast, other studies have reported a poor prognosis and more severe disease in patients with PBC and anti-gp210 antibodies. These disparate findings may be explained by the

ethnic origins or geographic locations of patients with PBC or the different antigenic preparations and assays used for anti-gp210 antibody detection [16, 17].

In 2001, Invernizzi et al assessed the clinical significance of antibody reactivity to NPC proteins using proteins purified from cellular extracts [66] and found that patients with PBC with detectable anti-NPC antibodies more frequently manifested cirrhosis. A subsequent study by the same group suggested that the presence of anti-NPC antibodies identified a group of patients that progressed faster and experienced a more unfavorable clinical course [64]. Nakamura et al [54] conducted a multi-center study based on retrospective cohorts of patients with PBC in the National Hospital Organization Study Group for Liver Disease in Japan and found that a sustained antibody response to the C-terminal immunodominant epitope of gp210 identified patients who were at higher risk for progression to end-stage liver disease. They further reported that patients who received ursodiol but retained high-titer serum antibodies to gp210 had a higher incidence of progression to end-stage liver disease [54].

The clinical significance of antibodies to gp210 have also been investigated in Japanese, British, Spanish, Italian and Greek cohorts of PBC and these studies have suggested that the their presence is associated with more severe disease and a worse prognosis [48, 56, 67, 68]. Nakamura et al [69] examined the expression of gp210 in livers and found significantly greater immunostaining for the protein in the NE of small bile ducts of subjects with PBC compared to controls. The same study reported that patients with greater portal inflammation, interface hepatitis and lobular inflammation expressed more gp210 in biliary epithelial cells [69]. These results led the authors to speculate that increased and/or aberrant expression of gp210 in small intrahepatic bile duct cells may contribute to their immune-mediated destruction. However, Nakamura et al [69] also noted increased gp210 immunostaining in 10–58% of specimens from patients without serum antibodies against gp210 who had chronic hepatitis B, chronic hepatitis C and autoimmune hepatitis, suggesting that over-expression of gp210 in biliary cells is not restricted to PBC. The authors subsequently reported in 276 patients with PBC that anti-gp210 antibodies were an independent risk factor for more severe interface hepatitis, lobular inflammation and ductular proliferation [53].

Anti-NPC antibodies after liver transplantation

Recurrence of PBC in the allograft after orthotopic liver transplantation has been well documented [70–73]. The persistence of PBC-specific AMA and ANA post-transplant has also been reported [50, 72, 73]. Mattalia and colleagues [50] tested patients for serum anti-gp210 autoantibodies before and after orthotopic liver transplantation and demonstrated that these autoantibodies persisted in the majority of patients after receiving allografts. A subsequent study by Luettig et al largely confirmed these findings, finding that gp210 autoantibodies may decrease in titer after transplantation but generally persist even after more than six years [52]. The lower antibody titers may result from intensive post-transplant immunsuppression. In a French series of patients with PBC who underwent orthotopic liver transplantation, the majority of patients had anti-gp210 antibodies six years afterwards [74].

Do anti-NPC antibodies arise by molecular mimicry?

A long-standing hypothesis about autoantibody generation in autoimmune diseases is that it is due to molecular mimicry. Shimoda et al [75] investigated the possible role of molecular mimicry between nuclear and mitochondrial antigens recognized by T-cells in PBC and identified sequences of various nuclear antigens, including gp210, sharing motifs of glutamate-any amino acid-aspartate-lysine or glutamate-isoleucine-glutamate-any amino acid-aspartate, which were also essential for the recognition of the major CD4 T-cell epitope

on the E2 subunit of mitochondrial pyruvate dehydrogenase complex (PDC-E2), the major target antigen of AMA [75]. The immunodominant PDC-E2 peptide was used to generate six reactive T cell clones, five of which reacted with gp210 mimics with prominent reactivity involving a peptide spanning amino acids 188–201 [75]. Shimoda et al also established three CD4 T-cell clones selected by recombinant gp210 and found that the epitope recognized by one clone was also reactive with the 188–201 peptide [75]. These results suggest that anti-gp210 autoantibodies may arise from molecular mimicry of PDC-E2 in PBC.

CONCLUSIONS

Autoantibodies against gp210, an integral membrane protein of the nuclear pore complex, are very specific for PBC. Autoantibodies against nup62, a non-membrane nucleoporin, also occur in the disease. Some studies suggest that these autoantibodies may identify individuals with PBC who have more severe disease and a poorer outcome; however, prospective studies are lacking. It remains unclear whether the loss of immunological tolerance and the induction of immune responses to these NPC antigens is an epiphenomenon, subsequent to cell destruction during an autoimmune assault, or primarily involved in the tissue pathogenesis of PBC.

TAKE-HOME MESSAGES

* Loss of immunological tolerance to the nucleus is amongst the best studied topics in autoimmunity.
* The diversity of the repertoire that reflect ANAs is often disease specific.
* In primary biliary cirrhosis, ANAs react with unique components of the nuclear envelope.
* Understanding the organization of the membranous structure of the nuclear envelope is critical for defining the specificity of ANAs in PBC.
* Anti-gp210 autoantibodies in PBC primarily recognize a 15 amino acid stretch within the C terminal domain.

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Abbreviations

AMA	antimitochondrial antibodies
ANA	antinuclear antibodies
IIF	indirect immunofluorescence microscopy
NE	nuclear envelope
NPC	nuclear pore complex
Nup	nucleoporin
PBC	primary biliary cirrhosis
PDC-E2	E2 subunit of mitochondrial pyruvate dehydrogenase complex

SLE systemic lupus erythematosus

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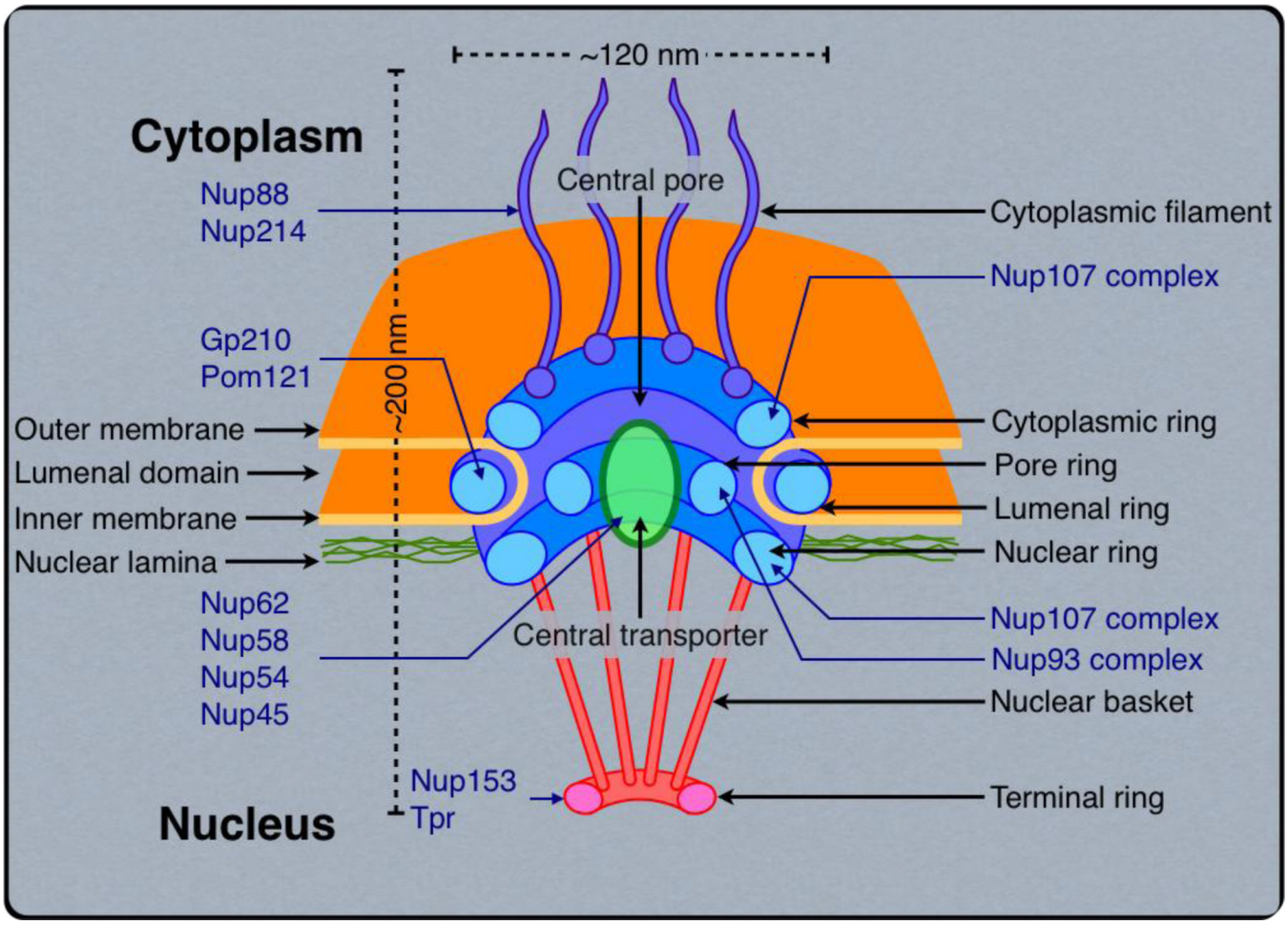

Figure 1.
Schematic diagram of a nuclear pore complex showing its major substructures and known localizations of several nucleoporins.