Antinuclear antibodies specific for primary biliary cirrhosis

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Abstract

The serological hallmark of primary biliary cirrhosis (PBC) is the presence of antimitochondrial antibodies. However, antinuclear antibodies (ANA) are also detectable in approximately 50% of subjects with PBC. Most clinical laboratories use indirect immunofluorescence microscopy to detect ANA and two labeling patterns that predominate in PBC are ‘punctate nuclear rim’ and ‘multiple nuclear dots.’ Work over the past several years has shown that antibodies giving these patterns most often recognize nuclear pore membrane protein gp210 and nuclear body protein sp100, respectively. These ANA are highly specific for PBC and detected in approximately 25% of patients. Less frequently, ANA apparently unique to PBC recognize other proteins of the nuclear envelope and nuclear bodies. While antibodies against gp210, sp100 and some other nuclear proteins are very specific to PBC and may therefore be useful diagnostic markers, their connection to pathogenesis remains to be elucidated.

Keywords: Antibodies; Primary biliary cirrhosis; Pathogenesis

1. Introduction

Primary biliary cirrhosis (PBC) has been considered to be a paradigmatic autoimmune disease [1]. While its pathogenesis remains obscure, considerable progress has been made in characterizing the autoantibody response that occurs in PBC. Recent results using recombinant human antigens have shown that nearly all patients with PBC have autoantibodies that recognize the E2 subunits of mitochondrial 2-oxo-acid dehydrogenase complexes [2], making the presence of antimitochondrial antibodies (AMA) the predominant serological feature of the disease. Approximately 50% of individuals with PBC also have antinuclear antibodies (ANA). Two particular autoantibodies, those that recognize nuclear pore membrane protein gp210 and those against nuclear body protein sp100, appear to be highly specific and detectable in approximately 25% of individuals with PBC. ANA against other nuclear envelope and nuclear body proteins also occur less frequently but appear to be highly specific for PBC.
2. Anti-gp210 antibodies

The nuclear pore membranes are annular, transmembranous structures associated with the nuclear pore complexes that connect the inner and outer nuclear membranes [3]. Nuclear pore complexes are macromolecular structures responsible for nucleocytoplasmic transport, including the import into the nucleus of proteins and the export to the cytoplasm of ribosomal and messenger RNAs. Several integral proteins of the pore membranes are components of the pore complexes. Antibodies against nuclear pore complex components were first suspected in sera from patients by analysis using immunofluorescence microscopy. Antibodies against nuclear pore complex components label the nuclear periphery in characteristic manners (Fig. 1). If the microscopic focal plane is on the top of the sperical interphase nucleus and the largest diameter is out of focus, a punctated pattern of the nuclear surface, with each dot representing a nuclear pore complex, is observed. If the focal plane is in the largest diameter of the interphase nucleus, a punctated, discontinuous perinuclear rim is seen, as only the nuclear pores are labeled. During mitosis, when the pore complexes disassemble and the nuclear membranes lose their identity and are absorbed by the endoplasmic reticulum from which they originate [3], the staining for pore complexes becomes only cytoplasmic.

In 1985, Ruffati et al. [4] reported that 18 of 63 patients with PBC had autoantibodies that labeled the nuclear periphery of fixed interphase cells examined using immunofluorescence microscopy, while only 1 of more than 400 serum samples from control patients gave a similar result. Subsequently, Lassoued et al. [5,6] and Lozano et al. [7] demonstrated that autoantibodies from patients with PBC that label the nuclear periphery reacted with a protein that had an apparent molecular mass of approximately 200 kDa. In 1990, the recognized antigen was identified as nuclear pore membrane protein gp210 [8]. As expected, gp210 autoantibodies from individuals with PBC give the characteristic labeling when examined by immunofluorescence microscopy (Fig. 1). However, in most cases, the perinuclear labeling may be obscured by the signal from AMA in the same serum, making careful observation necessary.

Gp210 is an integral protein localized to the nuclear pore membranes [9]. Mammalian gp210 has an amino-terminal domain of 1808 amino acids (after cleavage of its signal sequence) that is located in the perinuclear space, which is a continuation of the endoplasmic reticulum lumen, a single transmembrane segment and a carboxy-terminal ‘tail’ of 58 amino acids that faces the nuclear pore complex [10,11]. The luminal domain has several N-linked glycosylation modifications [9–11]. The ‘tail’ domain is phosphorylated in mitosis, possibly by cyclinB-p34cdc2 kinase [12]. In 1993, most anti-gp210 antibodies from patients with PBC were shown to recognize a stretch of only 15 amino acids in the protein’s carboxy-terminal ‘tail’ that faces the nuclear pore complex [13]. Subsequently, two studies using enzyme-linked immunoabsorbent assays with either a recombinant protein expressed in bacteria [14] or a chemically synthesized polypeptide [15] confirmed this result. In another study [16] using a portion of gp210 generated by protease digestion, autoantibodies from some patients with PBC were also shown to recognize a glycosylated fragment in the amino-terminal, luminal domain.

In six different studies [6,7,15–18], with samples sizes from 35 to 285, the prevalence of anti-gp210 antibodies in patients with a diagnosis of PBC has ranged from 9.5 to 41%. Based on these studies, the mean prevalence is approximately 25%. The antibodies generally persist after orthotopic liver transplantation, despite the fact that histological evidence of PBC is not present in the allografts [18–20]. More remarkable is the high specificity of anti-gp210 antibodies for PBC, which appears to be greater than 99% [5–7,15–17]. Therefore, the presence of anti-gp210 antibodies can be used to confirm the diagnosis of PBC in unusual cases, including those in which AMA may be present at only low titers or, in rare instances, undetectable (Fig. 1).

There are no long-term studies on the prognostic significance of anti-gp210 autoantibodies in PBC. Patients with or without detectable anti-gp210 antibodies appear to have similar clinical characteristics. In one study [6], subjects with gp210
Fig. 1. Indirect immunofluorescence microscopy using human HeLa cells and the serum of a patient with PBC directed against gp210 of the nuclear pore complex. HeLa cells were fixed with methanol, labeled with the serum of a patient with PBC and examined using a conventional microscope. The upper row shows pictures of a nucleus taken at two different plane of focus. In the left panel, the focal plane is on the top of the nucleus and the largest diameter is out of focus. Note the punctated pattern of the nuclear surface, with each dot representing a nuclear pore complex. In the right panel, the focal plane is in the largest diameter of the nucleus. Note that the perinuclear rim is discontinuous and punctated, as only the nuclear pores are labeled. The lower panel shows, on the right hand side the top of a nucleus and on the left hand side a cell in metaphase. During mitosis, nuclear membranes lose their identity and are absorbed by the endoplasmic reticulum from which they originate. The staining in metaphase is therefore only cytoplasmic and the shape of the mitotic spindle and chromosomes mass, which are unlabelled, are visible in the center of the cell. The particular serum used for these images is unusual as it is monospecific for gp210. Most of the sera from patients with PBC contain AMA, which stain the cytoplasm of the interphasic cells. Bar indicates 5 μm.
autoantibodies had a lower incidence of several associated conditions such as Raynaud’s phenomenon and arthralgias. In another study [17], subjects with PBC and detectable gp210 autoantibodies had a higher incidence of associated arthritis. The practical clinical implication of having detectable gp210 autoantibodies in PBC, if any, remains to be established.

3. Anti-sp100 antibodies

Nuclear bodies are multiprotein complexes that have been observed in all reported mammalian cell lines [21]. Approximately 10–30 bodies are observed per nucleus, ranging in size from 0.2 to 1 μm. The functions of the nuclear bodies are not clear but they may somehow be involved in cell differentiation or growth. Several proteins have been localized to nuclear bodies, arguably the most notable being promyelocytic leukemia (PML) protein [21]. When examined by immunofluorescence microscopy, antibodies against nuclear body proteins label ‘multiple nuclear dots’ in the nucleus.

In 1987, Szostecki et al. [22] showed that a predominant antigen recognized by autoantibodies from patients with PBC that produced a ‘multiple nuclear dot’ pattern recognized a protein with an apparent molecular mass of approximately 100 kDa they called sp100. Subsequently, the sp100 antigen was characterized by complementary DNA cloning [23]. Two other studies [24,25] have confirmed that the antigen recognized by autoantibodies from patients with PBC that give the ‘multiple nuclear dot’ pattern on immunofluorescence microscopy recognize a protein with a molecular mass of approximately 100 kDa. Anti-sp100 antibodies from patients with PBC recognize at least three non-overlapping domains of the protein and two stretches of 16–20 amino acids may be the predominant autoepitopes [26,27].

In three different studies [25,28,29], with sample sizes from 33 to 170, the prevalence of anti-sp100 antibodies in patients with a diagnosis of PBC has ranged from 18 to 44%. Based on these studies, their mean prevalence in PBC would be roughly 20–30%. The antibodies generally persist after orthotopic liver transplantation, despite the fact that histological evidence of PBC is not present in the allografts [19,20]. Anti-sp100 antibodies appear to be highly specific for a diagnosis of PBC and have only been detected in very few subjects without the disease [23,25,28,29]. Therefore, like anti-gp210 antibodies, the presence of anti-sp100 antibodies could be useful clinically to confirm the diagnosis of PBC in unusual cases. Their prognostic or clinical significance beyond this, if any, remains to be established.

4. Other ANA in PBC

Individuals with PBC also have a wide range of ANA that recognize different nuclear proteins. Some, such as those against centromere proteins, histones, spliceosome components and single stranded DNA reported in individuals with PBC [30], are not specific for the disease. Antibodies against nuclear lamins, which are also found in patients with several different autoimmune diseases [31,32], are also detected in approximately 2% of patients with PBC [6,15].

Some other nuclear envelope and nuclear body antigens other than gp210 and sp100 are also recognized by autoantibodies that are found almost exclusively in individuals with PBC, albeit less frequently. Rare patients with PBC have antibodies against LBR, an integral protein of the inner nuclear membrane [33]. LBR autoantibodies are anti-idiotypic to some antibodies against B-type nuclear lamins, a protein to which LBR binds [34]. The prevalence of LBR antibodies in individuals with PBC appears to be between 1 and 2% and these autoantibodies have not been reported in any other disease [15,17]. Anti-LBR antibodies in PBC recognize the first 60 amino acids of the protein [35].

PML is a protein of nuclear bodies that is associated with sp100 [21]. Antibodies against PML are often seen in individuals with PBC and anti-sp100 antibodies; however, they appear to be more difficult to detect. Using an immunoprecipitation assay with radiolabeled PML and immunofluorescence microscopy on cells that overexpressed the protein, Sternsdorf et al. [36] found anti-PML antibodies in most patients with PBC who had anti-sp100 antibodies. In a series of 170 patients with PBC, Zuchner et al. [28] reported
the prevalence of anti-PML antibodies to be 19%. A nuclear body protein called sp140 has also been identified and characterized using serum antibodies from a patient with PBC [37]. The prevalence of anti-sp140 antibodies in PBC is not yet known.

Two reports [38,39] have shown that antibodies in sera from individuals with PBC recognize a protein with a molecular mass of 62 kDa in a nuclear pore complex enriched fraction and claimed that appears to be nucleoporin p62. In one of these studies [38], the prevalence of antibodies of this specificity was reported to be 14% in patients with PBC. Antibodies against p62 have not been reported in other series that searched for antibodies against nuclear envelope proteins [5,6,15] and recognition of recombinant p62 has apparently not been reported.

5. ANA and the pathogenesis of PBC

Although not detectable in all patients, antibodies against gp210, sp100 and some other nuclear proteins are found almost exclusively in individuals with PBC. Their high specificity suggests that they may be involved in the disease pathogenesis. Unfortunately, no studies have been able to link either these autoantibodies or the antigens they recognize to the pathology of PBC. Convincing experimental data as to how or why ANA of these specificities are generated in individuals with PBC are also lacking. At this point in time, it can only be said that the connection of specific ANA to the pathogenesis of PBC remains to be elucidated.

6. Summary

ANA that recognize nuclear pore membrane protein gp210 and nuclear body protein sp100, while detectable in only a minority of patients, are highly specific for PBC. Several studies have suggested that other nuclear envelope and nuclear body proteins are also recognized specifically by autoantibodies from patients with PBC. Based on the number of studies performed and the work done on characterizing the autoantibodies, for example demonstrating reactivity with recombinant protein or identification of specific reactive epitopes, we summarize the specific ANA in PBC as definite or probable in Table 1. Because of their high specificity for PBC, detection of antibodies against the antigens listed in Table 1 may be clinically useful in diagnosis, especially in rare cases in which AMA are not detectable. Their clinical significance beyond their general utility in diagnosis has not been established. The connection of these antibodies to pathogenesis also remains to be elucidated.

Table 1

<table>
<thead>
<tr>
<th>Nuclear antigen recognized</th>
<th>Approximate prevalence in PBC (%)</th>
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<tbody>
<tr>
<td><strong>Definite</strong></td>
<td></td>
</tr>
<tr>
<td>gp210</td>
<td>25</td>
</tr>
<tr>
<td>sp100</td>
<td>20–30</td>
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<tr>
<td>LBR</td>
<td>2</td>
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<tr>
<td><strong>Probable</strong></td>
<td></td>
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<tr>
<td>PML</td>
<td>20</td>
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<tr>
<td>p62</td>
<td>14</td>
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Those listed as ‘definite’ have been reported in several different studies by different groups and/or rigorously characterized using recombinant antigenic epitopes. Those listed as ‘probable’ have been reported in fewer studies and less well characterized.

Take-home messages

- ANA occur in approximately 50% of individuals with PBC
- Antibodies against nuclear pore membrane protein gp210 are found in approximately 25% of individuals with PBC and are highly specific for the disease
- Antibodies against nuclear body protein sp100 are found in 20–30% of individuals with PBC and are highly specific for the disease
- Several other specific autoantibodies, such as those against inner nuclear membrane protein LBR and nuclear body protein PML, occur in some patients with PBC
- The relevance of specific ANA to prognosis and particular clinical features in PBC remains to be established
The connection of specific ANA to the pathogenesis of PBC is not known.

References

The World of Autoimmunity; Literature Synopsis

**Autoantibodies to steroidogenic enzymes in premature ovarian failure**

The prevalence of adrenal cortex autoantibodies, steroid-producing cell autoantibodies and autoantibodies to steroidogenic enzymes were compared in 3 groups of patients with premature ovarian failure. Among patients having premature ovarian failure and Addison’s disease, 73% were positive for steroid-producing cell antibodies, 93% were positive for 17alpha-hydroxylase and/or P450 side-chain cleavage enzyme autoantibodies, 93% were positive for adrenal cortex autoantibodies, and 100% had detectable antibodies to 21-hydroxylase enzyme. Among the other 2 groups of patients, those with either isolated premature ovarian failure or associated with other autoimmune diseases, these autoantibodies were detected in a much lower frequency. There were 2 patients with isolated premature ovarian failure and positive antibodies to adrenal cortex and 21-hydroxylase who developed Addison’s disease 3 to 5 years after ovarian failure onset. Hence, the presence of Addison’s disease in patients having premature ovarian failure is characterized by the presence of adrenal cells and steroidogenic enzymes autoantibodies (Dal Pra et al., Eur J Endocrinol 2003;148:565).


