Identification and Characterization of Autoantibodies Against the Nuclear Envelope Lamin B Receptor from Patients with Primary Biliary Cirrhosis

By Jean-Claude Courvalin,* Kaiss Lassoued,‡ Howard J. Worman,* and Günter Blobel*

*From the *Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, New York, New York 10021; and the ‡Laboratoire d'Immunochimie et Immunopathologie, Institut National de la Santé et de la Recherche Médicale (INSERM), U.108, Hôpital St. Louis, 75475 Paris, Cedex 10, France*

Summary

We have identified autoantibodies from two patients with primary biliary cirrhosis (PBC) that recognize the nuclear envelope of mammalian cells on indirect immunofluorescence microscopy. These antibodies bind to a 58-kD integral membrane protein (p58) of the turkey erythrocyte nuclear envelope, which has been previously identified as a membrane receptor for lamin B (Worman, H. J., J. Yuan, G. Blobel, and S. D. Georgatos. 1988. *Proc. Natl. Acad. Sci. USA.* 85:8531). The antibodies also bind to a 61-kD integral membrane protein (p61) of the rat liver nuclear envelope. Affinity-purified antibodies eluted from turkey p58 bind to rat p61, showing that the two proteins share an epitope(s) and that p61 is likely the rat liver lamin B receptor. In human nuclear envelopes, the antigen recognized has an apparent molecular mass close to that of avian protein. These findings, along with the previous discovery of autoantibodies against an integral membrane glycoprotein (gp210) of the nuclear pore membrane in patients with PBC, suggest that antibodies against integral membrane proteins of the nuclear envelope are characteristic of a subset of patients with PBC.

The nuclear membranes consist of three domains that are morphologically distinct from each other (for reviews see references 1 and 2). The outer nuclear membrane domain contains attached ribosomes and, at many points, is continuous with the rough endoplasmic reticulum (RER).[1] The nuclear pore membrane domain, which connects the outer and inner nuclear membranes and forms transcisternal circular pores, contains attached pore complexes. The inner nuclear membrane domain is attached to the nuclear lamina (3, 4), a structure composed of intermediate filament-type proteins, the acidic B-type lamins and the neutral A-type lamins (5–8). Although integral membrane proteins synthesized in the RER can reach the inner nuclear membrane (9), presumably by lateral diffusion via the pore membranes, it appears that integral membrane proteins are not randomly distributed between each of the three envelope domains. Instead each domain appears to contain specific integral membrane proteins. The pore membrane contains a recently characterized integral membrane glycoprotein gp210, that presumably functions in the attachment of the nuclear pore complex (10, 11). The inner membrane contains a number of unique proteins (12–14), among them a receptor for lamin B (14).

A number of human autoimmune diseases have been associated with high titers of autoantibodies against the nuclear envelope. These antibodies yield characteristic nuclear rim staining on immunofluorescence microscopy. Some of the antigens with which these autoantibodies react have been identified. For example, antibodies against A-type and B-type lamins have been described in systemic lupus erythematosus (15–17), autoimmune hepatitis (17, 18), and scleroderma (19). Autoantibodies against gp210, an integral membrane glycoprotein of the pore membrane domain, have been reported in patients with primary biliary cirrhosis (PBC) (20–22). Here we report, also in patients with PBC, autoantibodies against the lamin B receptor, an integral membrane protein of the inner nuclear membrane.

Materials and Methods

Indirect Immunofluorescence Microscopy. Detailed methods for immunofluorescence have been previously published (16). HeLa cells grown on cover slips were permeabilized for 6 min in methanol at −20°C. Antibodies from patient 1 (see below) were used at a 1:500 dilution. Second antibodies were affinity-purified goat

[1] *Abbreviations used in this paper:* PBC, primary biliary cirrhosis; RER, rough endoplasmic reticulum.

J. Exp. Med. © The Rockefeller University Press • 0022-1007/90/09/0961/07 $2.00
Volume 172 September 1990 961–967

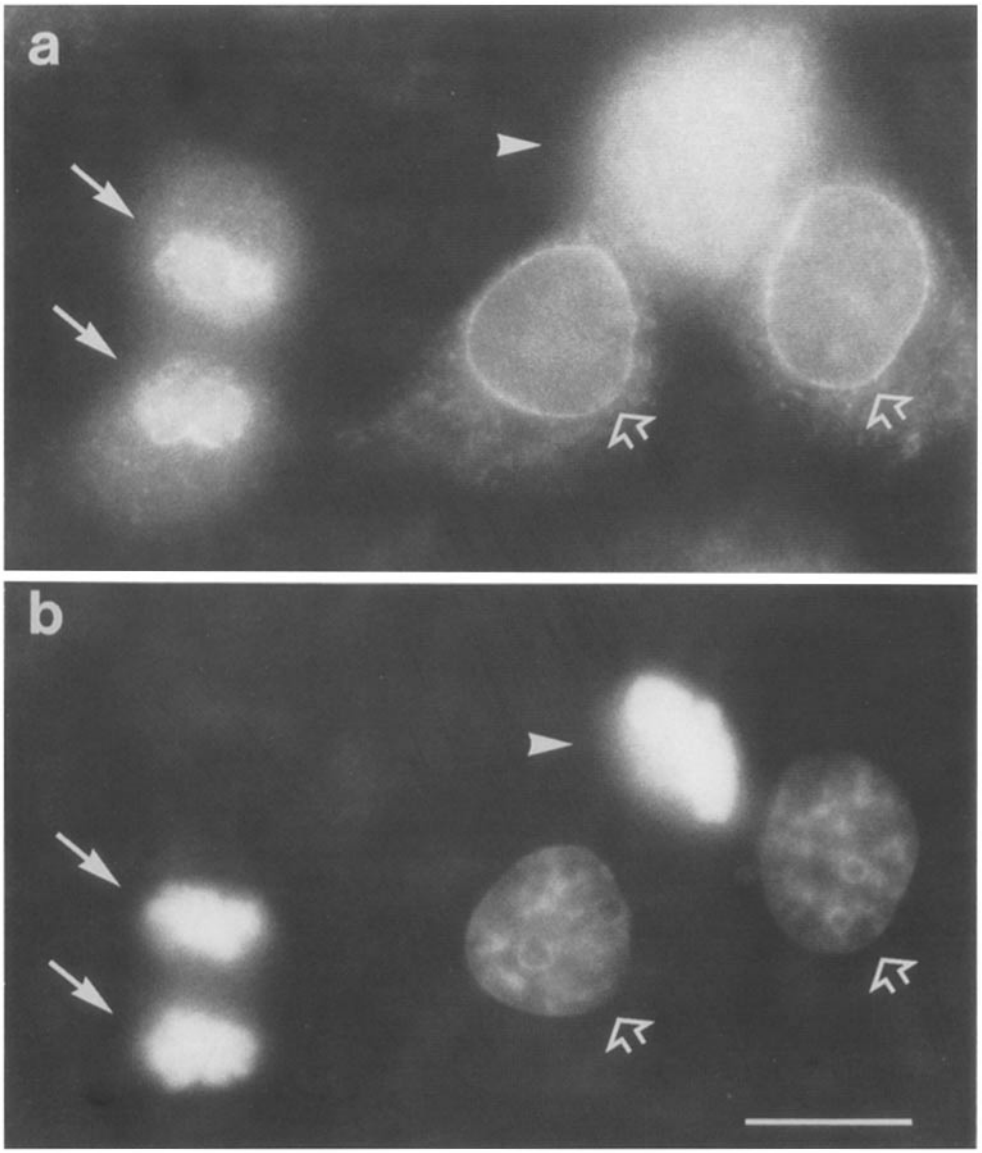

Figure 1. Indirect immunofluorescence microscopy of HeLa cells with the autoimmune antibodies. (*a*) Immunofluorescence micrograph using a 1:500 dilution of serum of patient 1 (see Materials and Methods); (*b*) staining of DNA of same cells with the fluorescent dye Hoechst 33258. Shown are two interphase cells (*open arrowheads*), one cell in metaphase (*closed arrowhead*) and two cells in telophase (*arrows*). Bar, 10 mm.

$F(ab')_2$ anti-human IgG γ chain FITC conjugate (Tago Inc., Burlingame, CA) used at a 1:100 dilution.

Preparation of Nuclei and Nuclear Envelope Fractions. Nuclei, nuclear envelope, and nuclear envelope subfractions were prepared from rat liver as previously described (22). Nuclear envelopes from turkey erythrocytes were prepared according to Georgatos and Blobel (23). Nuclei from HeLa cells were prepared as described earlier (20) and nuclear envelopes were prepared from these nuclei as described for rat liver nuclei except that the DNase I/RNase A digestion step at 4°C was for 3 h instead of 1 h and the last centrifugation step was performed without a sucrose cushion.

Immunoblotting. SDS-solubilized proteins were subjected to electrophoresis under reducing conditions on polyacrylamide gels according to Laemmli (24). Proteins were transferred to nitrocellulose sheets by electrophoresis using a semi-dry method (25). All the following steps were carried out in 10 mM Tris-HCl (pH 7.4), 0.15 M NaCl, 0.1% Tween-20, and 50 g/liter non-fat milk (Carnation, Los Angeles, CA). Nitrocellulose strips were blocked for 1 h, incubated for 2 h with the indicated dilution of serum, washed four times, incubated with a 1:2,000 dilution of ^{125}I-protein A (New England Nuclear, Wilmington, DE), washed four more times, and then exposed to x-ray film at −70°C with intensifying screens.

Affinity Purification of Antibodies. Turkey erythrocyte nuclear envelopes were extracted with urea as described (23) and immunoblotted with the patient's serum diluted 1:100. The band corresponding to the 58-kD lamin B receptor was excised and antibodies eluted according to Smith and Fisher (26). A band of the same blot in the 150-kD region was eluted under the same conditions and the eluate used as control.

Antibodies. Autoimmune antibodies were detected by indirect immunofluorescence microscopy on air-dried rat liver tissue sections. Both sera gave continuous perinuclear staining up to a dilution of 1:500. Monospecific rabbit IgG specific for human Ig heavy and light chains (Dako, Kobenhaven, Denmark) indicated that the antibodies were polyclonal IgG and IgM molecules.

Guinea pig polyclonal antibodies against turkey p58 have been previously described (14). The following human autoimmune sera were used as controls: two sera directed against gp210, four an-

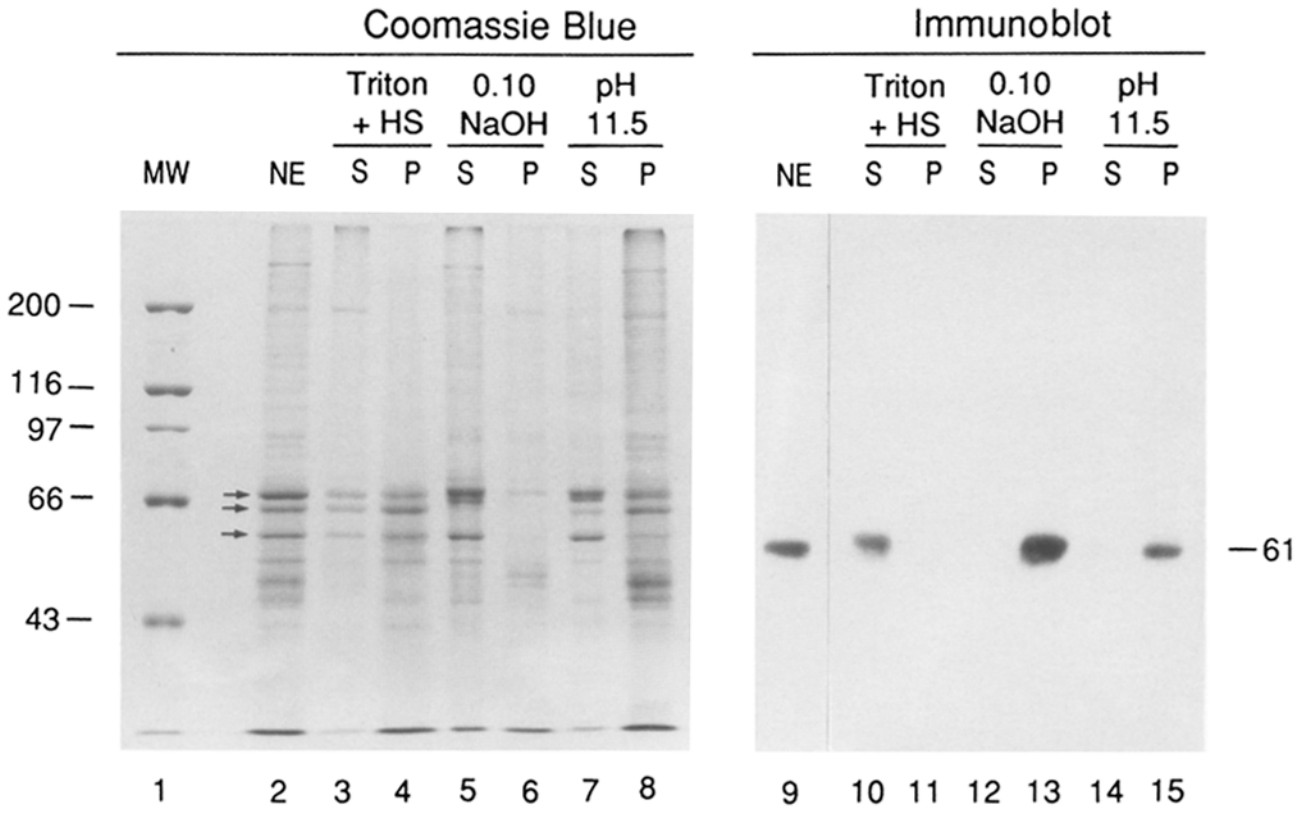

Figure 2. Fractionation behavior of the 61-kD antigen upon extraction of rat liver nuclear envelopes with Triton X-100/NaCl or alkaline solutions. Nuclear envelopes were incubated with either 2% Triton X-100/0.3 M NaCl, 0.1 M NaOH, or Na_2CO_3 (pH 11.5) (as indicated in the figure) and centrifuged to yield supernatant (S) and pellet (P) fractions. Polypeptides in these fractions or of unfractionated nuclear envelopes (NE), were separated by SDS-PAGE and visualized by Coomassie blue staining (lanes *1–8*) or were transferred to nitrocellulose (lanes *9–15*) and probed with a 1:200 dilution of the serum of patient 1. Lane *1* contains molecular mass markers. Arrows in left panel refer to lamins A (74 kD), B (68 kD), and C (60 kD).

tisera directed against lamins, and two sera without antinuclear antibodies. All of these sera were previously characterized (17, 20, 22).

Patients. Sera were obtained from two patients. Patient 1 was a 36-yr-old woman with PBC diagnosed by liver biopsy. She had no associated rheumatic symptoms and no antimitochondrial antibodies. Patient 2 was a 59-yr-old woman with PBC and associated Sjögren's syndrome. The diagnosis was determined by liver biopsy. She did not have antimitochondrial antibodies but her serum did contain antibodies against histones.

Results

Autoantibodies Stain the Nuclear Envelope. Indirect immunofluorescence microscopy of HeLa cells fixed at different stages of the cell cycle with the serum of patient 1 yielded a staining pattern that is characteristic for components of the nuclear envelope (27, 28). Interphase cells showed staining of the nucleus enhanced at the nuclear rim (Fig. 1 *a*, *open arrowheads*). Metaphase cells containing disassembled components of the nuclear envelope throughout the cytoplasm showed a diffuse staining of the entire cell (Fig. 1 *a*, *closed arrowheads*). Telophase cells with the nuclear envelope mostly reassembled showed again intense staining largely coincident with the condensed daughter chromosomes (Fig. 1 *a*, *arrows*).

The Autoantigen Is an Integral Membrane Protein. To determine the molecular mass of the reactive autoantigen(s), we isolated rat liver nuclear envelopes and separated their proteins by SDS-PAGE. The separated proteins were stained with Coomassie blue (Fig. 2, lanes *2*), or transferred to nitrocellulose sheets and probed with the serum of patient 1. A polypeptide with an apparent molecular mass of 61 kD was found to react with the autoantibodies (Fig. 2, lane *9*).

To determine whether the 61-kD polypeptide is a peripheral or integral membrane protein, rat liver nuclear envelopes were extracted with either 0.1 M NaOH, or 0.1 M $NaCO_3$ at pH 11.5 or were solubilized by a solution of 2% Triton X-100/0.3M NaCl. The 61-kD polypeptide was resistant to alkaline extraction (Fig. 2, lanes *12–15*) and therefore fractionates as an integral membrane protein. As expected for an integral membrane protein, it was solubilized by a solution of detergent and high salt (Fig. 2, lanes *10* and *11*).

The Autoantigen Is the Previously Identified Lamin B Receptor. Several integral membrane proteins of molecular masses similar to the 61-kD antigen have recently been identified in the inner nuclear membrane (13, 14). Among these is the lamin B receptor of nuclear envelopes from avian erythrocytes with an apparent M_r of 58 kD (14). Using guinea pig antibodies against the avian lamin B receptor (p58) (14), we

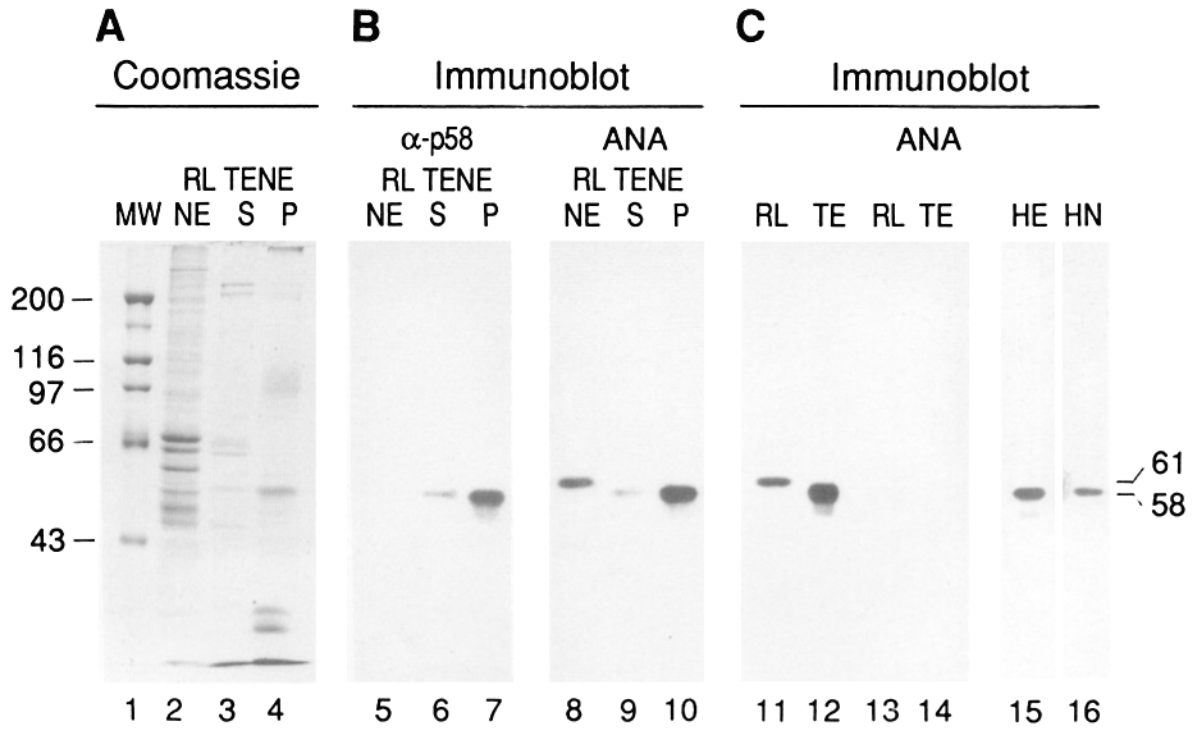

Figure 3. The 61-kD rat and the 59-kD human antigen are homologues of the 58-kD lamin B receptor of turkey. Polypeptides in total rat liver nuclear envelopes (RLNE) and supernatants (S) and pellets (P) of urea incubated turkey erythrocyte nuclear envelopes (TENE) were separated by SDS-PAGE and either visualized by Coomassie blue staining (*A*, lanes *2–4*), or transferred to nitrocellulose (*B*, lanes *5–7* and *8–10*) and probed with either the guinea pig antiserum (1:500 dilution) against the turkey lamin B receptor (α-p58) (lanes *5–7*) or with serum of patient 1 diluted 1:200 (ANA) (lanes *8–10*). In *C*, polypeptides of rat liver nuclear envelopes (RL) and pellets from urea extracted turkey erythrocytes nuclear envelopes (TE) were subjected to SDS-PAGE, transferred to nitrocellulose, and probed with affinity-purified antibodies of the antiserum of patient 1, using turkey p58 as a ligand (lanes *11*, *12*). Lanes *13* and *14* correspond to control antibodies (see Materials and Methods). Immunoblots of SDS-PAGE resolved polypeptides of HeLa cell nuclear envelopes (HE) and of crude HeLa nuclei (HN) revealed with serum of patient 1 are shown in lanes *15* and *16*, respectively. The numbers 61 and 58 on the right of lane *16* indicate M_r in kD of lamin B receptor in different species.

compared the reactivity of these antibodies with that of the human autoantibodies using blots of SDS-PAGE separated polypeptides of avian (turkey erythrocytes) and mammalian (rat liver) nuclear envelopes (Fig. 3). As reported previously, the guinea pig antibodies reacted with p58 of avian erythrocyte nuclear envelopes most of which was not extracted by 8 M urea (Fig. 3, compare lanes *6* and *7*). The same serum did not react with any polypeptide of rat liver nuclear envelopes (Fig. 3, lane *5*). The serum of patient 1 reacted also with a 58-kD polypeptide of the avian nuclear envelopes that was not extracted by 8 M urea (Fig. 3, compare lanes *9* and *10*). Thus, by the criteria of M_r as well as resistance to extraction by 8 M urea, the human serum and the guinea pig anti-p58 antiserum recognized an identical polypeptide in the nuclear envelope of avian erythrocytes, indicating that this polypeptide contains epitope(s) that are recognized by both sera and, moreover, that the serum of patient 1 is directed against the lamin B receptor.

As the serum of patient 1 reacted with a single polypeptide of 61 kD of rat liver nuclear envelopes (Fig. 3, lane *8*) that, like p58 of turkey erythrocyte nuclear envelopes, is an integral membrane protein, it is likely that turkey p58 and rat p61 are homologous. To further substantiate this conclusion, we affinity purified the patient's serum using turkey p58 as a ligand and tested the affinity-purified antibodies on blots containing SDS-PAGE separated rat liver (Fig. 3, lane *11*) or turkey erythrocyte (Fig. 3, lane *12*) nuclear envelope proteins. The affinity-purified antibodies reacted with a 61-kD polypeptide in rat liver nuclear envelopes (lane *11*) and a 58-kD polypeptide in turkey erythrocyte nuclear envelopes (lane *12*). As a control, material that was eluted from proteins other than p58 of turkey erythrocyte nuclear envelopes (see Materials and Methods) did not react with any proteins of either rat liver (lane *13*) or turkey erythrocyte (lane *14*) nuclear envelopes.

We also tested the serum of patient 1 on nitrocellulose blots of SDS-PAGE separated polypeptides of a crude nuclear fraction of HeLa cells (Fig. 3, lane *16*) or with a nuclear envelope fraction derived from this nuclear fraction (Fig. 3, lane *15*). Again a single polypeptide reacted, but in these cells the M_r of the reactive protein was ~59 kD, less than the rat liver 61-kD protein but close to the turkey erythrocyte 58-kD protein.

As the autoimmune serum of patient 2 gave the identical immunofluorescence pattern (data not shown) as that observed for the autoimmune serum of patient 1, we tested both an-

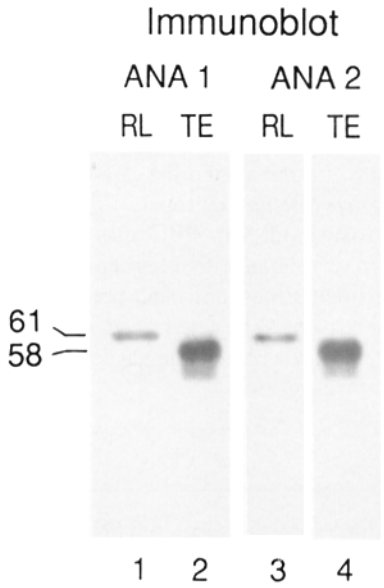

Figure 4. Immunoblot comparing the sera of patients 1 and 2. Immunoblots were performed with SDS-PAGE resolved polypeptides of either total rat liver (RL) nuclear envelopes (lanes *1* and *3*) or urea-extracted turkey erythrocyte (TE) nuclear envelopes (lanes *2* and *4*). Serum from patient 1 (ANA 1) was used in lanes *1* and *2* and serum from patient 2 (ANA 2) was used in lanes *3* and *4*. Molecular masses of the two immunoreactive components are indicated at the left of lane *1*.

tisera side by side on blots containing SDS-PAGE resolved polypeptides of rat liver and turkey erythrocyte nuclear envelopes (Fig. 4). As in the case of patient 1 (Fig. 4, lanes *1* and *2*), the autoimmune serum of patient 2 reacted with only a single protein of 61 kD in rat liver nuclear envelopes (Fig. 4, lane *3*) or of 58 kD in turkey erythrocyte nuclear envelopes (Fig. 4, lane *4*).

These sera were the only two of 66 directed against nuclear envelope proteins that recognized the rat liver 61-kD integral membrane protein. 24 of the sera examined were directed against nuclear lamins (17 and unpublished data) and 40 against gp210 (17, 20, 22, 29). Besides the two characterized in this study, eight of these sera used as controls in immunoblotting experiments of avian nuclear envelopes did not recognize the lamin B receptor (p58).

Discussion

Antinuclear antibodies are found in a wide variety of autoimmune diseases. Several antigens responsible for homogeneous, speckled, or nucleolar labeling of the nuclei have been identified (30). Because of the lower frequency of autoantibodies that stain the nuclear periphery, only a few of the antigens recognized by these antibodies have been identified. Among these are the A-type and B-type lamins associated with the inner nuclear membrane domain of the nuclear envelope. Antibodies against these proteins are found in patients with different autoimmune diseases (15–19). Recently, an integral membrane glycoprotein of the pore membrane domain of the nuclear envelope gp210 was identified as an antigen that is recognized by sera of some patients with PBC (22).

In the present study we have identified a new autoantigen of the nuclear envelope in two patients with PBC. This antigen is an integral protein of the inner nuclear membrane. On immunoblotting of rat liver nuclear envelopes, these antibodies recognized an integral membrane protein of molecular mass 61 kD, suggesting that the target antigen could be the lamin B receptor, a protein first identified in turkey erythrocyte nuclear envelopes with a similar molecular mass (14). The close similarity between these proteins was confirmed by the finding that the autoantibodies reacted with the avian lamin B receptor (p58). Antibodies affinity purified against turkey p58 were furthermore able to react with rat p61, demonstrating that an interspecies conserved epitope of the lamin B receptor was shared by the avian and rat proteins. The antibodies also reacted with a protein of similar molecular mass (~59 kD) in human nuclear envelopes, suggesting that cross-reactive proteins are present in all three of the species examined.

PBC is a chronic liver disease of unknown etiology. Histologically, PBC is characterized by progressive inflammatory destruction of the intrahepatic bile ducts with eventual progression to cirrhosis (31). Diagnosis is based on immunological and/or pathological criteria (31–33). The presence of antimitochondrial autoantibodies directed against a family of antigens termed M2 is characteristic of PBC (34). Several constituents of the M2 antigens have been identified (35–38) and the corresponding antibodies shown to be present in high titer and with a high frequency (90–95%) in patients with PBC. However, they are not specific for PBC and are sometimes present in other autoimmune diseases (39–41). Of note, the two patients characterized in this study did not have antimitochondrial antibodies.

Antinuclear antibodies are also found in patients with PBC. Antibodies to histones, which were present in the serum of patient 2 of this study, are present in 74% of patients with PBC (42), but are also found in association with other autoimmune diseases (30). Antibodies to gp210 (see above) are found in only 27% of the patients with PBC, but they are likely highly specific for this disease since they have not yet been found associated with other diseases (29). So far antibodies to the lamin B receptor also appear to be disease specific, as they have not yet been detected in any published series in which samples were screened from patients with other autoimmune diseases or liver diseases. However, more antibodies directed against the lamin B receptor have to be analyzed to assess the prevalence and the specificity of this new immunological marker. Thus, it appears that the two families of autoantibodies directed against integral membrane proteins of the nuclear envelope are found exclusively in PBC. In contrast, autoantibodies against the nuclear lamins, which are peripheral proteins of the nuclear envelope, have been mainly reported in patients with lupus-like syndromes and autoimmune hepatitis (15–18). Why antibodies to integral membrane proteins of the nuclear envelope are restricted to patients with PBC is not known.

Epitope(s) recognized by immunoglobulins in a few autoimmune diseases including PBC have recently been identified

(43–45). As the cloning and sequencing of the cDNA for p58 has been recently completed (46), this identification will now be possible for this protein. This is of importance since autoantibodies in this study are directed against conserved domain(s) of the lamin B receptor which may be of functional significance. Data suggest that autoantibodies to the lamin B receptor reported here are antiidiotypic to some lamin B autoantibodies (Lassoued, K., F. Danon, and J.C. Brouet, manuscript submitted for publication). Thus the conserved epitope(s) that is the target of these anti-bodies could be the binding site of the lamin B receptor to lamin B.

The role of autoantibodies in the etiology and pathogenesis of autoimmune diseases is not clear. For example, the role of mitochondrial antigens in the origin of PBC has recently been questioned. Immunization of experimental animals with one of the identified antigens, the human purified recombinant pyruvate dehydrogenase (PDH-E2), generated antimitochondrial antibodies but not PBC (47). Moreover, postimmunization and autoimmune antibodies against this protein were found to recognize different epitopes, suggesting that antimitochondrial autoantibodies in PBC may be the result of specific breakdown of tolerance to a few epitope(s) (48). Because similar experiments have not been performed with the lamin B receptor, it remains to be determined whether autoantibodies to this protein play a role in the pathogenesis of PBC in these patients.

We thank Drs. F. Danon, J.-C. Brouet, S. Georgatos, and W. Reeves for helpful discussion and Ms. Bargis-Touchard for secretarial assistance. Cet article est dédié à Thomas.

This research was supported in-part by a fellowship from The North Atlantic Treaty Organization to Dr. J.-C. Courvalin (INSERM) and a Physician-Scientist Award (grant DK-01790) from The National Institutes of Health to Dr. H. Worman.

Address correspondence to Dr. J.-C. Courvalin, Laboratory of Cell Biology, Howard Hughes Medical Institute, The Rockefeller University, Box 292, 1230 York Avenue, New York, NY 10021. J.-C. Courvalin's permanent address is Institut J. Monod du CNRS. Université Paris 7, 2 Place Jussieu, 75251 Paris Cedex 05, France. H. J. Worman's present address is Department of Medicine, Division of Liver Diseases, The Mount Sinai School of Medicine, One Gustav L. Levy Place, New York, NY 10029.

Received for publication 29 May 1990 and in revised form 18 June 1990.

References

1. Newport, J.W., and D.J. Forbes. 1987. The nucleus: structure, function, and dynamics. *Annu. Rev. Biochem.* 56:335.
2. Gerace, L., and B. Burke. 1988. Functional organization of the nuclear envelope. *Annu. Rev. Cell Biol.* 4:335.
3. Fawcett, D.W. 1966. On the occurrence of a fibrous lamina on the inner aspect of the nuclear envelope in certain cells of vertebrates. *Am. J. Anat.* 119:129.
4. Gerace, L., and G. Blobel. 1982. Nuclear lamina and the structural organization of the nuclear envelope. *Cold Spring Harbor Symp. Quant. Biol.* 46:967.
5. McKeon, F.D., M.W. Kirschner, and D. Caput. 1986. Homologies in both primary and secondary structure between nuclear envelope and intermediate filament proteins. *Nature (Lond.).* 319:463.
6. Fisher, D.Z., N. Chaudhary, and G. Blobel. 1986. cDNA sequencing of nuclear lamins A and C reveals primary and secondary structural homology to intermediate filament proteins. *Proc. Natl. Acad. Sci. USA.* 83:6450.
7. Krohne, G., S.L. Wolin, F.D. McKeon, W.W. Franke, and M.W. Kirschner. 1987. Nuclear lamin L1 of *Xenopus laevis*: cDNA cloning, amino acid sequence and binding specificity of a member of the lamin B subfamily. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3801.
8. Höger, T.H., G. Krohne, and W.W. Franke. 1988. Amino acid sequence and molecular characterization of murine lamin B as deduced from cDNA clones. *Eur. J. Cell Biol.* 47:283.
9. Torrisi, M.R., L.V. Lotti, A. Pavan, G. Migliaccio, and S. Bonatti. 1987. Free diffusion to and from the inner nuclear membrane of newly synthesized plasma membrane glycoproteins. *J. Cell Biol.* 104:733.
10. Gerace, L., Y. Ottaviano, and C. Kondor-Koch. 1982. Identification of a major polypeptide of the nuclear pore complex. *J. Cell Biol.* 95:826.
11. Wozniak, R.W., E. Bartnik, and G. Blobel. 1989. Primary structure analysis of an integral membrane glycoprotein of the nuclear pore. *J. Cell Biol.* 108:2083.
12. Richarson, J.C.W., and A.H. Maddy. 1980. The polypeptides of nuclear envelope: II. Comparison of rat liver nuclear membrane polypeptides with those of the rough endoplasmic reticulum. *J. Cell Sci.* 43:269.
13. Senior, A., and L. Gerace. 1988. Integral membrane proteins specific to the inner nuclear membrane and associated with the nuclear lamina. *J. Cell Biol.* 107:2029.
14. Worman, H.J., J. Yuan, G. Blobel, and S.D. Georgatos. 1988. A lamin B receptor in the nuclear envelope. *Proc. Natl. Acad. Sci. USA.* 85:8531.
15. Reeves, W.H., N. Chaudhary, A. Salerno, and G. Blobel. 1987. Lamin B autoantibodies in sera of certain patients with systemic lupus erythematosus. *J. Exp. Med.* 165:750.
16. Guilly, M.N., F. Danon, J.C. Brouet, M. Bornens, and J.C. Courvalin. 1987. Autoantibodies to nuclear lamin B in a patient with thrombopenia. *Eur. J. Cell Biol.* 43:266.
17. Lassoued, K., M.N. Guilly, F. Danon, C. André, D. Dhumeaux, J.P. Clauvel, J.C. Brouet, M. Seligmann, and J.C. Cour-

valin. 1988. Antinuclear autoantibodies specific for lamins: characterization and clinical significance. *Ann. Intern. Med.* 108:829.
18. Wesierska-Gadek, J., E. Penner, E. Hichman, and G. Sauermann. 1988. Antibodies to nuclear lamins in autoimmune liver disease. *Clin. Immunol. Immunopathol.* 49:107.
19. McKeon, F.D., D.L. Tuffanelli, K. Fukuyama, and M. Kirschner. 1983. Autoimmune response directed against conserved determinants of nuclear envelope proteins in a patient with linear scleroderma. *Proc. Natl. Acad. Sci. USA.* 80:4374.
20. Lassoued, K., M.N. Guilly, C. André, M. Paintrand, D. Dhumeaux, F. Danon, J.C. Brouet, and J.C. Courvalin. 1988. Autoantibodies to 200 kD polypeptide(s) of the nuclear envelope: a new serologic marker of primary biliary cirrhosis. *Clin. Exp. Immunol.* 74:283.
21. Lozano, F., A. Parès, L. Borche, M. Plana, T. Gallart, J. Rodès, and J. Vives. 1988. Autoantibodies against nuclear envelope-associated proteins in primary biliary cirrhosis. *Hepatology.* 8:930.
22. Courvalin, J.-C., K. Lassoued, E. Bartnik, G. Blobel, R.W. Wozniak. 1990. The 210 kilodalton nuclear envelope polypeptide recognized by human autoantibodies in primary biliary cirrhosis is the major glycoprotein of the nuclear pore. *J. Clin. Invest.* 85:279.
23. Georgatos, S.D., and G. Blobel. 1987. Lamin B constitutes an intermediate filament attachment site at the nuclear envelope. *J. Cell Biol.* 105:117.
24. Laemmli, U.K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680.
25. Kyhse-Andersen, J. 1984. Electroblotting of multiple gels: a simple apparatus without buffer tank for rapid transfer of proteins from polyacrylamide to nitrocellulose. *J. Biochem. Biophys. Methods.* 10:203.
26. Smith, D.E., and P.A. Fisher. 1984. Identification, developmental regulation, and response to heat shock of two antigenically related forms of a major nuclear envelope protein in Drosophila embryos: application of an improved method for affinity purification of antibodies using polypeptides immobilized on nitrocellulose blots. *J. Cell Biol.* 99:20.
27. Gerace, L., A. Blum, and G. Blobel. 1978. Immunocytochemical localization of the major polypeptides of the nuclear pore complex-lamina fraction. *J. Cell Biol.* 79:546.
28. Davis, L.I., and G. Blobel. 1986. Identification and characterization of a nuclear pore complex protein. *Cell.* 45:699.
29. Lassoued, K., R. Brenard, F. Degos, J.C. Courvalin, C. André, F. Danon, J.C. Brouet, Y. Zine-el-Abidine, C. Degot, S. Zafrani, D. Dhumeaux, and J.-P. Benhamou. 1990. Antinuclear antibodies directed to a 200 kD polypeptide of the nuclear envelope in primary biliary cirrhosis. A clinical and immunological study of a series of 150 patients with primary biliary cirrhosis. *Gastroenterology.* 99:181.
30. Tan, E.M. 1989. Antinuclear antibodies: diagnostic markers for autoimmune diseases and probes for cell biology. *Adv. Immunol.* 44:93.
31. Kaplan, M.M. 1987. Primary biliary cirrhosis. *N. Engl. J. Med.* 316:521.
32. Sherlock, S., and P.J. Scheuer. 1973. The presentation and diagnosis of 100 patients with primary biliary cirrhosis. *N. Engl. J. Med.* 289:674.
33. Christensen, E., J. Crowe, D. Doniach, H. Popper, L. Ranek, J. Rodes, N. Tygstrup, and R. Williams. 1980. Clinical pattern and course of disease in primary biliary cirrhosis based on an analysis of 236 patients. *Gastroenterology.* 78:236.
34. Lindenborn-Fotinos, J., H. Baum, and P.A. Berg. 1985. Mitochondrial antibodies and their relation to primary biliary cirrhosis: species and nonspecies specific determinants of M2 antigens. *Hepatology.* 5:763.
35. Coppel, R.L., L.J. McNeilage, C.D. Surh, J. Van de Water, T.W. Spithill, S. Whittingham, and M.E. Gershwin. 1988. Primary structure of the human M2 mitochondrial autoantigen of primary biliary cirrhosis: dihydrolipoamide acetyltransferase. *Proc. Natl. Acad. Sci. USA.* 85:7317.
36. Fussey, S.P.M., J.R. Guest, O.F.W. James, M.F. Bassendine, and S.J. Yeaman. 1988. Identification and analysis of the major M2 autoantigens in primary biliary cirrhosis. *Proc. Natl. Acad. Sci. USA.* 85:8654.
37. Surh, C.D., D.J. Danner, A. Ahmed, R.L. Coppel, I.R. Mackay, E.R. Dickson, and M.E. Gershwin. 1989. Reactivity of primary biliary cirrhosis sera with a human fetal liver cDNA clone of branched chain alpha-keto acid dehydrogenase Dihydrolipoamide acetyltransferase, the 52 kD mitochondrial antigen. *Hepatology.* 9:63.
38. Fregeau, D.R., P.A. Davis, D.J. Danner, A. Ansari, R.L. Coppel, E.R. Dickson, and M.E. Gershwin. 1989. Antimitochondrial antibodies of primary biliary cirrhosis recognize dihydrolipoamide acyltransferase and inhibit enzyme function of the branched chain alpha-ketoacid dehydrogenase complex. *J. Immunol.* 142:3815.
39. Mouritsen, S., E. Demant, H. Permin, and A. Wilk. 1986. High prevalence of anti-mitochondria antibodies among patients with some well-defined connective tissue diseases. *Clin. Exp. Immunol.* 60:68.
40. Munoz, L.E., H.G. Thomas, P.J. Scheur, D. Doniach, and S. Sherlock. 1981. Is mitochondrial antibody diagnostic of primary biliary cirrhosis? *Gut.* 22:136.
41. Berg, P.A., and R. Klein. 1987. Immunology of primary biliary cirrhosis. *Baill. Clin. Gastroenterology.* 1:675.
42. Penner, E., S. Muller, D. Zimmerman, and M.H.V. van Regenmortel. 1987. High prevalence of antibodies to histones among patients with primary biliary cirrhosis. *Clin. Exp. Immunol.* 70:47.
43. Chambers, J.C., and J.D. Keene. 1985. Isolation and analysis of cDNA clones expressing human lupus La antigen. *Proc. Natl. Acad. Sci. USA.* 82:2115.
44. Lieu, T.S., M.M. Newkirk, J.D. Capra, and R.D. Sontheimer. 1988. Molecular characterization of human Ro/SS-A antigen. *J. Clin. Invest.* 82:96.
45. Van de Vater, J., M.E. Gershwin, P. Leung, A. Ansari, and R.L. Coppel. 1988. The autoepitope of the 74-kD mitochondrial autoantigen of primary biliary cirrhosis corresponds to the functional site of dihydrolipoamide acetyltransferase. *J. Exp. Med.* 167:1791.
46. Worman, H.J., C.D. Evans, and G. Blobel. 1990. The lamin B receptor of the nuclear envelope inner membrane: a polytopic protein with eight potential transmembrane domains. *J. Cell Biol.* In press.
47. Krams, S.M., C.D. Surh, R.L. Coppel, A. Ansari, B. Ruebner, and M.E. Gershwin. 1989. Immunization of experimental animals with dihydrolipoamide acetyltransferase, as a purified recombinant polypeptide, generates mitochondrial antibodies but not primary biliary cirrhosis. *Hepatology.* 9:411.
48. Surh, C.D., A. Ahmed-Ansari, and M.E. Gershwin. 1990. Comparative epitope mapping of murine monoclonal and human autoantibodies to human PDH-E2, the major mitochondrial autoantigen of primary biliary cirrhosis. *J. Immunol.* 144:2647.