

## Early reports

# Detection of retroviral antibodies in primary biliary cirrhosis and other idiopathic biliary disorders

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## Summary

**Background** Retroviruses have been implicated in the aetiology of various autoimmune diseases. We used immunoblots as a surrogate test to find out whether retroviruses play a part in the development of primary biliary cirrhosis.

**Methods** We did western blot tests for HIV-1 and the human intracisternal A-type particle (HIAP), on serum samples from 77 patients with primary biliary cirrhosis, 126 patients with chronic liver disease, 48 patients with systemic lupus erythematosus, and 25 healthy volunteers.

**Findings** HIV-1 p24 gag seroreactivity was found in 27 (35%) of 77 patients with primary biliary cirrhosis, 14 (29%) of 48 patients with systemic lupus erythematosus, 14 (50%) of 28 patients with chronic viral hepatitis, and nine (39%) of 23 patients with either primary sclerosing cholangitis or biliary atresia, compared with only one (4%) of 24 patients with alcohol-related liver disease or  $\alpha_1$ -antitrypsin-deficiency liver disease, and only one (4%) of 25 healthy volunteers ( $p=0.003$ ). Western blot reactivity to more than two HIAP proteins was found in 37 (51%) of patients with primary biliary cirrhosis, in 28 (58%) of patients with systemic lupus erythematosus, in 15 (20%) of patients with chronic viral hepatitis, and in four (17%) of those with other biliary diseases. None of the 23 patients with either alcohol-related liver disease or  $\alpha_1$ -antitrypsin deficiency, and only one of the healthy controls showed the same reactivity to HIAP proteins ( $p<0.0001$ ). Our results showed a strong association between HIAP seroreactivity and the detection of

autoantibodies to double-stranded DNA. HIAP seroreactivity was also strongly associated with the detection of mitochondrial, nuclear, and extractable nuclear antigens.

**Interpretation** The HIV-1 and HIAP antibody reactivity found in patients with primary biliary cirrhosis and other biliary disorders may be attributable either to an autoimmune response to antigenically related cellular proteins or to an immune response to uncharacterised viral proteins that share antigenic determinants with these retroviruses.

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## Introduction

The hypothesis that primary biliary cirrhosis has an infectious component is supported by reports of clustering of the disorder among families and care workers, the increased prevalence within the distribution of a particular water supply,<sup>1</sup> and recurrence after liver transplantation, as shown by detection of serum antimitochondrial antibody to the mitochondrial antigen pyruvate dehydrogenase complex E2 (PDC-E2), granulomatous destruction of bile ducts, and immunohistochemical evidence of an antigen resembling PDC-E2 on biliary epithelial cell surfaces, a feature specific to primary biliary cirrhosis.<sup>2,3</sup>

A preliminary report of false-positive HIV-1 ELISA results for a proportion of patients with primary biliary cirrhosis<sup>4</sup> is interesting because similar serological findings have been reported in patients with Sjögren's syndrome and systemic lupus erythematosus.<sup>5,6</sup> The human intracisternal A-type particle (HIAP) has been isolated from the salivary glands of Sjögren's syndrome patients.<sup>7</sup> Other endogenous human retroviruses have been identified and implicated in the aetiology of insulin-dependent diabetes mellitus and multiple sclerosis.<sup>8,9</sup> Western blot studies have shown that the majority of patients with Sjögren's syndrome and systemic lupus erythematosus have strong reactivity to characterised HIAP proteins, whereas only a minority of disease controls and healthy individuals show the same reactivity.<sup>7,10</sup> Since primary biliary cirrhosis and these multisystem disorders share clinical features, we did HIV-1 and HIAP western blot studies to find out whether patients with primary biliary cirrhosis show evidence of retroviral infection.

## Patients and methods

We used stored serum samples from 77 primary biliary cirrhosis patients, and serum samples from a comparison group of 126 patients with other liver diseases. Samples were

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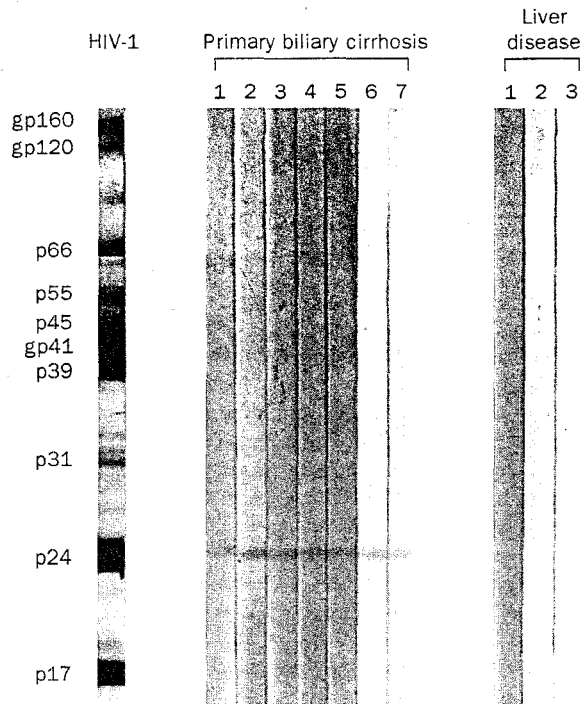


Figure 1: Western blot analysis of serum incubated with HIV-1 proteins

HIV-1=HIV-1 serum (positive control).

collected from academic tertiary referral centres in New Orleans and Philadelphia between 1987 and 1996. 24 patients in the comparison group had no infectious or biliary disease: 16 had alcohol-related liver disease, and eight had  $\alpha_1$ -antitrypsin deficiency. The remaining comparison samples were from 19 patients with primary sclerosing cholangitis, four patients with biliary atresia, 46 patients with chronic hepatitis B virus (HBV) infection, and 33 patients with chronic hepatitis C virus (HCV) infection. We also studied, as controls, serum from 48 patients with systemic lupus erythematosus, at the Rheumatology Clinic at Tulane Medical Center School of Medicine, and 25 healthy volunteers from the same institution. Research permission was granted by the Alton Ochsner Medical Foundation and the Tulane Medical Center review boards.

For the immunoblotting of HIV-1 nitrocellulose strips (Cambridge Biotech, Cambridge, MA, USA) we used 20  $\mu$ L serum at a dilution of 1 in 100.<sup>36</sup> Serum from a patient with HIV-1 infection was used as the positive control. HIV-1 western blots were judged to be positive if there was reactivity with p24 or p31 as well as either gp41 or gp160 envelope proteins.

The microsomal fraction was obtained from both HIAP-I-infected and uninfected RH9/MC lymphoblastoid cell lines by disruption of cells in a hypotonic buffer followed by density ultracentrifugation over a 33–68% sucrose gradient.<sup>10,11</sup> Fractions were resolved by sodium dodecyl

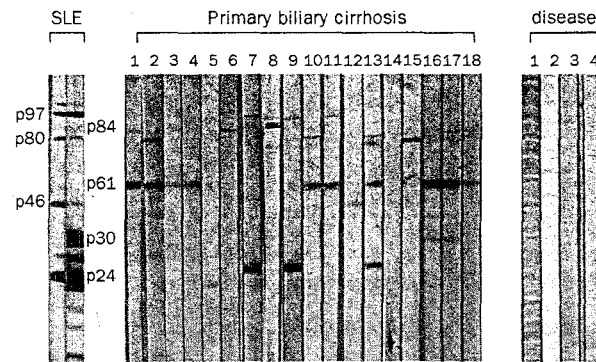


Figure 2: Western blot analysis of serum incubated with HIAP proteins

SLE=systemic lupus erythematosus.

sulphate/polyacrylamide gel electrophoresis, blotted onto nitrocellulose, and used for immunoblotting studies by incubation of 10  $\mu$ L serum at a dilution of 1 in 400. HIAP western blots were judged to be positive if there was reactivity to two or more known HIAP proteins.<sup>7,10–12</sup> Selected samples were assayed in duplicate to check reproducibility. Serum from ten primary biliary cirrhosis patients with positive HIAP western blots was analysed with immunoblots from uninfected RH9 cells, to show specific reactivity to HIAP proteins. Antibodies derived by screening a primary biliary cirrhosis combinatorial immunoglobulin library with PDC-E2 were also used for the HIAP and HIV-1 immunoblot studies, to assess these specific antimitochondrial antibodies for cross-reactivity with retroviral proteins.<sup>13</sup>

All the serum samples from the 126 patients with liver diseases were also screened for IgG autoantibody reactivity at Specialty Laboratories (Santa Monica, CA, USA). Antimitochondrial antibodies, antinuclear antibodies, and antiribosomal antibodies were assessed by microscopy with an image analyser, after immunofluorescence labelling of either HEP-2000 slides (Immuno Concepts, Irvine, CA, USA) or rat tissue. Extractable nuclear antigen reactivity, anticardiolipin reactivity, and Sjögren's syndrome A and B reactivity were detected by EIA, and the Farr assay was used to detect antibodies to double-stranded DNA.<sup>14–16</sup>

Our statistical analysis used the Pearson's  $\chi^2$  test. Fisher's exact test was used when more than 20% of the expected values in our frequency tables were lower than 5.

## Results

No patient with liver disease showed reactivity to the HIV-1 gag and envelope proteins; the established criteria for HIV-1 infection. Immunoblot reactivity was generally restricted to p24 and, in a few cases, p17 in the patients with liver disease (figure 1). The frequency of HIV-1 p24 gag reactivity in the patients with primary biliary cirrhosis was not significantly different from that in the groups with systemic lupus erythematosus,

Comparison groups	n	Number with immunoblot reactivity					
		p24	p*	$\geq 1$ HIAP	p*	$\geq 2$ HIAP	p*
Healthy	25	1 (4%)	0.003	2 (8%)	<0.0001	1 (4%)	<0.0001
ALD or $\alpha_1$ -AT	24	1 (4%)	0.003	1 (4%)	<0.0001	0	<0.0001
HBV infection	43	9 (60%)*	0.07	14 (33%)	<0.0001	5 (12%)	<0.0001
HCV infection	33	5 (38%)*	0.8	19 (58%)	0.1	10 (30%)	0.04
PSC or BA	23	9 (39%)	0.42	9 (39%)	0.002	4 (17%)	0.004
SLE	46	14 (29%)	0.49	37 (77%)	0.66	28 (58%)	0.35
Primary biliary cirrhosis	77	27 (35%)	..	53 (75%)	..	37 (51%)*	..

ALD=alcohol-induced liver disease.  $\alpha_1$ -AT= $\alpha_1$ -antitrypsin deficiency. PSC=primary sclerosing cholangitis. BA=biliary atresia. SLE=systemic lupus erythematosus.

\*For comparison with primary biliary cirrhosis.

†Data not available for 28 HBV-infected and 20 HCV-infected patients because tests not done.

‡Data not available for 6 patients because tests not done.

Table 1: Frequency of HIV-1 p24 and HIAP immunoblot reactivity in patients with primary biliary cirrhosis and comparison groups

Comparison groups	n	Autoantibody reactivity (n)						
		AMA	ANA	Anti-dsDNA	Anti-ENA	Anti-SSA/B	Anticardiolipin	Antiribosomal
PSC or BA	23	0	2 (9%)	4 (17%)	3 (13%)	ND	3 (13%)	1 (4%)
HBV infection	46	1 (2%)	4 (9%)	2 (4%)	0	0	24 (%)	1 (2%)
HCV infection	33	0	1 (3%)	2 (6%)	3 (9%)	0	26 (%)	0
ALD or $\alpha_1$ -AT	21	0	3 (14%)	0	3 (14%)	ND	1 (5%)	0
<b>Primary biliary cirrhosis</b>	<b>77</b>	<b>74 (96%)</b>	<b>19 (27%)</b>	<b>35 (45%)</b>	<b>9 (12%)</b>	<b>5 (6%)</b>	<b>68 (%)</b>	<b>2 (3%)</b>

ALD=alcohol-induced liver disease,  $\alpha_1$ -AT= $\alpha_1$ -antitrypsin deficiency, PSC=primary sclerosing cholangitis, BA=biliary atresia, ND=not done.

AMA=antimitochondrial antibody; ANA=antinuclear antibody; dsDNA=double-stranded DNA; ENA=extractable nuclear antigen; SSA/B=Sjögren's syndrome A and B.

Table 2: Frequency of IgG autoantibodies in all patients with chronic liver diseases

chronic viral hepatitis, or other biliary disorders, but it was significantly higher than the frequencies among the healthy volunteers and patients with alcohol-related liver disease or  $\alpha_1$ -antitrypsin deficiency (table 1).

A proportion of patients with systemic lupus erythematosus had pronounced reactivity to all known p17, p24, p30, p46, p60, p80, p84, and p97 HIAP proteins (figure 2) as determined by previous western blot studies.<sup>7,10-12</sup> In the patients with primary biliary cirrhosis, the HIAP immunoblot reactivity was generally less pronounced; no patient showed reactivity to all HIAP proteins, but p61 reactivity was commonly found (figure 2). Duplicate western blots revealed reproducible results; none of the HIAP-positive samples tested had serum reactivity to immunoblots from uninfected RH9 cells. In the retroviral immunoblot studies with combinational antibodies specific for PDC-E2 and biliary epithelial cells from patients with primary biliary cirrhosis, none of the three antibodies bound to HIV-1 or HIAP proteins.

The frequency of HIAP western blot reactivity did not differ significantly between the primary biliary cirrhosis and systemic lupus erythematosus groups, but there were significant differences between the primary biliary cirrhosis group and the liver-disease comparison groups (table 1).

Autoantibody reactivity in the patients with chronic liver disease (table 2) was in accordance with previous

reports.<sup>16-18</sup> In the analysis of retroviral and autoantibody reactivity, HIAP reactivity was more common in autoantibody-positive patients than in autoantibody-negative patients within each subgroup (table 3). No link between HIV-1 p24 and autoantibody reactivity was evident, except for patients with anticardiolipin, among whom frequency of HIV-1 p24 reactivity was higher than that in patients without the autoantibody ( $p=0.03$ ).

## Discussion

We used HIV-1 and HIAP western blot tests to show that many of our patients with primary biliary cirrhosis had seroreactivity with retroviral proteins. Although no immunoblot reactivity was found with the PDC-E2 combinational antibodies,<sup>13</sup> there were significant associations with HIAP reactivity and with the presence of various antibodies. Similar autoantibody profiles have been found in patients with HIV-1 infection, leading researchers to speculate that autoantibody production may be related to antigenic similarities between HIV-1 and host proteins.<sup>10</sup>

Our findings suggest opposing hypotheses for the generation of retroviral antibodies in patients with primary biliary cirrhosis. If the disorder is purely autoimmune, without an infectious element, patients will make autoantibodies to their own cellular constituents. These autoantibodies may coincidentally cross-react with shared antigenic determinants of viral

Autoantibody reactivity	Serum reactivity								
	HIV-1 p24 gag			≥1 HIAP*			≥2 HIAP*		
	Positive	Negative	p	Positive	Negative	p	Positive	Negative	p
<b>Antimitochondrial antibody</b>									
Positive	25	49		50	18		33	35	
Negative	26	52	0.95	46	80	<0.0001	23	103	<0.0001
<b>Antinuclear antibody</b>									
Positive	7	20		21	7		11	17	
Negative	44	77	0.42	75	90	0.004	45	120	0.19
<b>Anti-double-stranded DNA</b>									
Positive	13	26		29	12		18	23	
Negative	35	70	0.83	67	88	0.002	38	115	0.02
<b>Anti-extractable nuclear antigen</b>									
Positive	5	10		14	4		10	8	
Negative	46	88	0.94	82	94	0.01	46	130	0.01
<b>Anti-SSA/B</b>									
Positive	1	4		2	3		2	1	
Negative	40	64	0.41	50	67	1.00	50	99	0.27
<b>Anticardiolipin</b>									
Positive	8	8		10	4		5	9	
Negative	43	88	0.03	86	94	0.08	51	129	0.56
<b>Antiribosomal</b>									
Positive	2	1		4	0		2	2	
Negative	49	97	0.25	92	96	0.06	54	136	0.30

SSA/B=Sjögren's syndrome A and B.

\*Reactivity to either #1 or #2 characterised p17, p24, p30, p46, p60, p80, p84, and p97 HIAP proteins.

† $\chi^2$  analysis or Fisher's exact test, comparing frequency of retroviral reactivity with seroprevalence of autoantibodies.

Table 3: Autoantibody prevalence compared with serum reactivity to HIV-1 p24 and HIAP in chronic liver disease patients

proteins. For example, in serological studies of systemic lupus erythematosus patients without HIV-1 infection, Talal and colleagues<sup>6</sup> have shown that the Sm ribonuclear protein, an extractable nuclear antigen, can inhibit binding to HIV-1 p24 gag. In reciprocal studies, HIV-1 p24 inhibited the binding of an idiotypic antibody to the Sm protein.<sup>6</sup>

An alternative hypothesis suggests that patients with primary biliary cirrhosis are infected with a virus that shares antigenic determinants with HIV-1 p24 gag and HIAP. In the past, researchers doing HIV-1 immunoblot studies thought that HIV-1 gag shared conserved antigenic determinants with other retroviral nucleocapsid proteins.<sup>3</sup> These studies showed that HIV-1 p24 gag reactivity was present in 30–35% of patients with Sjögren's syndrome and systemic lupus erythematosus, compared with only 5% of those with adult rheumatoid arthritis, 2% of patients with polymyositis, and less than 1% of healthy individuals.<sup>5,6,10</sup> More recently, researchers have isolated HIAP from RH9 lymphoblastoid cells cocultured with cells from the salivary glands of patients with Sjögren's syndrome, by use of an antigen-capture ELISA with HIV-1 p24 gag antibodies, on the assumption that HIAP shares antigenic determinants with HIV-1 gag.<sup>7,10</sup> On electronmicroscopy, the virus resembled rodent intracisternal A-type particles. These particles are transmitted endogenously within the host genome, and exogenously as a defective particle.<sup>7,10</sup> HIAP western blot studies have shown that 90–95% of patients with Sjögren's syndrome and systemic lupus erythematosus and 87.5% of patients with Graves' disease have seroreactivity with HIAP, compared with less than 3% of patients with adult rheumatoid arthritis and 2% of healthy individuals.<sup>7,10–12</sup> These accumulated data suggest that HIV-1 and HIAP immunoblot assays may be suitable for use as specific, sensitive, surrogate assays for viral infection in patients with idiopathic autoimmune diseases.

In our study, patients with chronic viral hepatitis also had immunoreactivity to HIV-1 p24. This may be partly explained by the conserved conformational epitopes or shared immunodominant antigens common to HBV, HCV, and HIV-1. To date, HIAP aminoacid sequence data are not available for similar analysis, and there is no obvious explanation for the significant ( $p < 0.05$ ) differences in HIAP reactivity on western blot found between patients with HBV and HCV (table 1). Since patients with HCV infection can develop sialadenitis, and, rarely, Sjögren's syndrome, we speculate that HCV infection may modulate the expression of HIAP, which is classed as an endogenously encoded retroviral element.<sup>7,10,19,21</sup>

The detection of HIV-1 p24 and HIAP antibodies in patients with primary sclerosing cholangitis may give an insight into the pathogenesis of this idiopathic biliary disorder. If there is a link between sclerosing cholangitis and hereditary or acquired immunodeficiency syndromes, an infectious agent must play a major part in the pathogenesis of this biliary disease. The similar cholangiographic appearance of patients with primary sclerosing cholangitis and AIDS cholangiopathy also makes a strong case for a microbial pathogenesis of primary sclerosing cholangitis.<sup>7</sup> Even though patients with AIDS cholangiopathy are commonly infected with other microbial agents, such as cytomegalovirus and

protozoa, in some of these patients no pathogens other than HIV-1 can be found.<sup>22</sup>

In conclusion, our study suggests that many patients with primary biliary cirrhosis show antibody reactivity to proteins that share antigenic determinants with HIV-1 p24 or HIAP. The antibody reactivity may be the result of an autoimmune response to cross-reactive cellular proteins, or a response to infection with an uncharacterised viral element. The latter hypothesis is supported by the identification of retroviral agents in other autoimmune disorders.<sup>7–9</sup> Our study has implications for the management of primary biliary cirrhosis, and is a starting point for study of the links between viral infection and autoantibody production.

#### Contributors

Andrew Mason, Lizhe Xu, and Robert Garry planned the study. M Eric Gershwin, Yehuda Shoenfeld, Santiago Munoz, Jonathan Jaspán, and Michael Bryer-Ash contributed to the study design. Lizhe Xu, Linsheng Guo, Santiago Munoz, Yan Cao, David Sander, Judy Van de Water, Yehuda Shoenfeld, and Alaa Ahmed did the laboratory studies and data analysis. Santiago Munoz collected serum samples. Andrew Mason wrote the paper and Robert Garry, M Eric Gershwin, Yehuda Shoenfeld, and Michael Bryer-Ash gave critical review. Jonathan Jaspán died before the paper was submitted.

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## Congenital abnormalities in Brazilian children associated with misoprostol misuse in first trimester of pregnancy

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### Summary

**Background** Misoprostol is commonly used to induce abortion in Brazil, and in other countries in South and Central America where abortions are illegal. However, misoprostol is not very effective in inducing abortions, and exposure to the drug in utero can cause abnormalities in the fetus. We aimed to define the common phenotypical effects of exposure to the drug.

**Methods** We studied 42 infants from São Paulo, Brazil, who were exposed to misoprostol during the first 3 months of gestation, and then born with congenital abnormalities. We interviewed each of the infants' mothers to find out about misoprostol exposure and dosage. Each infant was physically examined by a geneticist or a neuropaediatrician.

**Findings** 17 of the infants had equinovarus with cranial-nerve defects. Ten children had equinovarus as part of more extensive arthrogryposis. The most distinctive phenotypes were arthrogryposis confined to the legs (five cases) and terminal transverse-limb defects (nine cases) with or without Möbius sequence. The most common dose of misoprostol taken was 800 µg (range 200-16 000 µg).

**Interpretation** Deformities attributed to vascular disruption were found in these children. We suggest that the uterine contractions induced by misoprostol cause vascular disruption in the fetus, including brain-stem ischaemia.

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Information on the effects of taking misoprostol during pregnancy should be made more widely available, to dissuade women from misusing the drug.

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### Introduction

In Brazil, and in other countries in South and Central America, misoprostol, a synthetic analogue of prostaglandin E, is commonly used to induce abortion.<sup>1,5</sup> Abortion is illegal in Brazil, except in cases of rape or incest, or when the mother's life is in danger. The demand for illegal abortions is high, and misoprostol can be bought from pharmacies and on the black market. However, misoprostol is not very effective at inducing abortion, and exposure to misoprostol in utero can cause abnormalities in the fetus. Thus, exposure to this teratogen is common in Brazil.

Misoprostol (Cytotec, Searle do Brasil) is marketed for treatment of upper-gastrointestinal damage caused by non-steroidal anti-inflammatory drugs. The manufacturer's label states that misoprostol is contraindicated for use in pregnant women, since it has uterotonic effects.<sup>6</sup> Publicity about the illegal use of misoprostol contrary to the manufacturers' instructions has led to the imposition of restrictions, and an 80% decrease in sales of the drug.<sup>7</sup> However, misoprostol is still available through illegal sources.

The first report of fetal damage from the unsuccessful use of misoprostol to induce abortion described unusually large lateral defects of the scalp and cranium in five infants.<sup>3</sup> Gonzalez and colleagues<sup>7</sup> reported seven children with terminal transverse-limb defects. These defects were commonly associated with cranial-nerve defects, also known as Möbius syndrome. Since case reports cannot prove a causal link between in-utero exposure to misoprostol and birth defects, systematic studies are needed to identify the phenotypic effects of misoprostol and to define the period of greatest sensitivity to the drug during pregnancy. Unfortunately, use of