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Prevalence of Autoantibodies to the p53 Protein in Autoimmune Hepatitis

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The target antigens of anti-nuclear autoantibodies in autoimmune hepatitis (AIH) are poorly characterised. Since antibodies to the p53 nuclear protein have been reported in various autoimmune diseases, we have assessed the prevalence of these antibodies in patients with AIH ($n = 45$), primary biliary cirrhosis ($n = 60$), hepatitis B ($n = 22$), hepatitis C ($n = 55$), and in a control group of subjects with various non-liver diseases ($n = 56$). A significant proportion of patients with AIH (31%) had elevated levels of autoantibodies to the p53 protein. In contrast, the prevalence of these antibodies in primary biliary cirrhosis (8%) and viral hepatitis (6%) was similar to that in the control group (4%). The clinical features of the anti-p53 seropositive AIH patients were similar to those of the seronegative ones. Thus, the prevalence of p53 autoantibodies in AIH is higher than in other forms of chronic hepatitis, and may be useful in differential diagnosis.

Keywords: Autoimmunity; p53; Inflammation; ANA; Hepatitis; AIH

INTRODUCTION

Autoantibodies to various autoantigens are the serological feature of autoimmune hepatitis (AIH)[1,2] and may contribute to the pathological process in this condition.[3] The most prevalent autoantibodies in AIH are anti-nuclear antibodies (ANA); however, the molecular targets of ANA in AIH are poorly defined. Identification of the nuclear antigens recognised by ANA may help in elucidating the pathological processes in AIH.

One of the candidate antigens for being a target of AIH-associated ANA is the p53 molecule, a tumour suppressor involved in DNA repair,[4] growth arrest and apoptosis.[5] Various forms of stress can induce the accumulation of the p53 protein in stressed cells: high levels of p53 have been found in tumours[6] and in inflammatory cells.[7,8] The accumulation of the p53 molecule seems to trigger p53 autoimmunity: tumour patients[9] and patients suffering from various chronic inflammatory autoimmune diseases,[10–12] including systemic lupus erythematosus (SLE)[13,14] and type I diabetes[15] exhibit elevated levels of anti-p53 antibodies. Anti-p53 antibody titers in patients with autoimmune diseases are lower than those in patients with cancer.[12] Moreover, the specificity of the p53 antibodies found in these populations differ: patients suffering from SLE recognise predominantly the carboxy-terminal DNA-binding domain of p53,[14] while tumour patients appear to recognise predominantly the amino-terminus of the p53 molecule.[16]

Since chronic inflammation of the liver and hepatocellular stress have been reported to be associated with accumulation of the p53 molecule,[17,18] we have hypothesised that AIH might be associated with autoantibodies to the p53 protein. We have, therefore, assessed the prevalence of autoantibodies to the p53 molecule and to the carboxy-terminal DNA-binding domain of the p53 protein in AIH and other inflammatory hepatic disease.

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EXPERIMENTAL PROCEDURES

Human Sera

Sera were obtained from 182 patients attending the liver out-patient clinic of the Johannes Gutenberg University, Mainz, Germany: 45 with AIH, 60 with PBC and 77 with chronic viral hepatitis (22 hepatitis B; 55 hepatitis C). As control, 56 sera were obtained from 9 healthy subjects and from 47 consecutive patients attending the general out-patient clinic of the Johannes Gutenberg University, Mainz, Germany, with various non-immunological and non-hepatic diseases (mainly trauma and cardiovascular patients). The control sera from the 47 general medical patients and from the 9 healthy subjects showed no apparent differences and were thus grouped together.

Antigens

E. Coli BL21 (DE3) cells were transformed with the pET30 expression vector (Novagen, Bad Schwalbach, Germany) containing human p53 cDNA; the His-tag provided by the vector was used for purification of recombinant p53 by Ni-chelate-chromatography (Qiagen, Hilden, Germany). The carboxy-terminal p53 DNA-binding domain peptide p363–382[19,20] was prepared using an automated synthesizer (Abimed AMS 422; Langenfeld, Germany) according to the manufacturer. Peptide purity was confirmed by analytical reverse phase HPLC and mass spectroscopic analysis. The sequence of the p53 carboxy peptide (p363–382) is: HSSYLKTKK-GQSTSRHKKTM.

Immunoassays

ELISA assays were done in 96-well Maxisorp plates (Nunc, Roskilde, Denmark), in which each well was coated with 10 μg/ml test antigen in PBS. After washing and blocking with 1% BSA in PBS for 1 h at 37°C, diluted test sera (1:200; 0.1 ml per well) were added for 1 h at 37°C and then incubated for 1 h with goat anti-human IgG specific secondary antibody conjugated to horseradish peroxidase (DAKO, Copenhagen Denmark), diluted 1:5000. A substrate solution containing 1 mg/ml ABTS in 40 mM citric acid, 60 mM Na_2HPO_4, and 3‰ H_2O_2 (all from Sigma, Taufkirchen, Germany) was added, and the plates were read at 405 nm. The test antigens used were recombinant p53, or the p53 carboxy-peptide p363–382. The data are given as the optical density (OD) values produced by the test sera. Seropositivity for antibodies to the p53 protein and to the p53 carboxy-peptide was defined as producing an OD value that was higher than the sum of the control group mean plus twice the standard deviation; thus, sera with an OD above 0.507 for the p53 protein and an OD above 0.32 for the p53 carboxy-peptide were considered positive.

HLA Testing

HLA alleles of patients were determined by standard serological and genetical haplotyping techniques at the centre for blood transfusion of the Johannes Gutenberg University, Mainz, Germany.

Statistics

The differences between experimental groups were tested for significance using the non-parametric Mann–Whitney test. Correlation coefficients were calculated using the non-parametric Spearman rank correlation analysis.

RESULTS

The sera of the 182 liver patients and the 56 control sera were tested for the presence of p53 autoantibodies. The control sera from the 47 general medical patients and from the 9 healthy subjects showed similar results and were thus grouped together.

Sera from patients with AIH had significantly higher levels of anti-p53 antibodies than control sera ($P = 0.0008$; Fig. 1); in contrast, reactivity to p53 in patients with PBC ($P = 0.9568$), hepatitis B ($P = 0.8376$) and hepatitis C ($P = 0.2526$) did not differ significantly from controls. Levels of antibodies to the carboxy-terminal DNA-binding domain of p53, as detected by reactivity to the p53 carboxy-peptide, were significantly higher in the AIH ($P = 0.0003$) and the hepatitis C

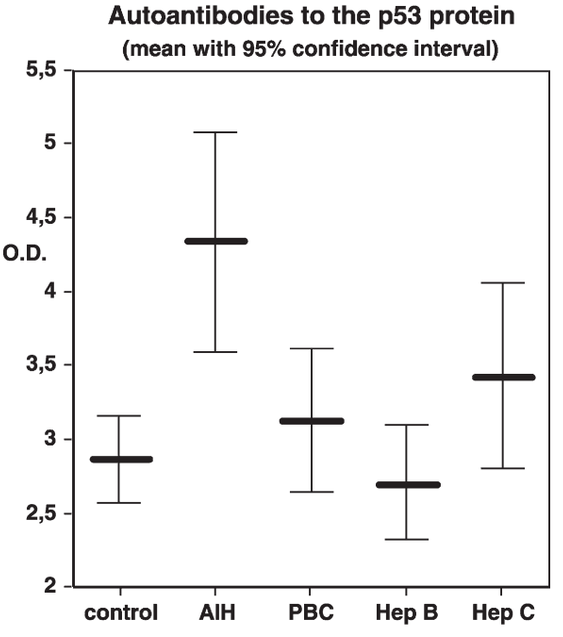

FIGURE 1 The serum levels of autoantibodies to the p53 molecule were determined by ELISA in subjects consecutively attending the general clinic (control), or suffering from autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), hepatitis B virus infection (Hep B) or hepatitis C virus infection (Hep C). Shown is the mean OD produced by the sera and the 95% confidence interval of the mean.

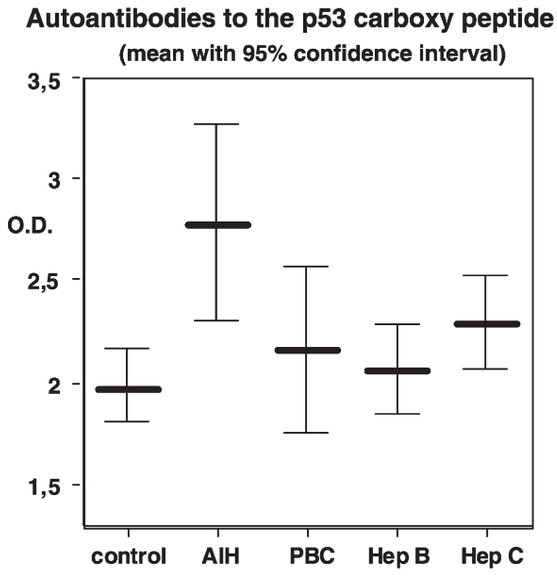

FIGURE 2 The serum levels of autoantibodies to the carboxy-terminal DNA-binding domain of the p53 molecule were determined by ELISA in subjects consecutively attending the general clinic (control), or suffering from autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC), hepatitis B virus infection (Hep B) or hepatitis C virus infection (Hep C). Shown is the mean OD produced by the sera and the 95% confidence interval of the mean.

patients ($P = 0.0133$) than in the PBC ($P = 0.9824$) and the hepatitis B patients ($P = 0.3836$), in whom levels did not differ from controls (Fig. 2). There was a positive correlation between levels of antibodies to the p53 protein and antibodies to the p53 carboxy-peptide (Table I) in the AIH ($r = 0.8727$), PBC ($r = 0.9162$) and hepatitis B patients ($r = 0.8771$). However, the prevalence of antibodies to p53 was significantly higher than in controls only in the AIH group (31% versus 4%, Table I). Only a few sera tested positive for anti-p53 autoantibodies among PBC (8%), hepatitis B (5%) and hepatitis C (7%) patients.

To investigate whether seropositivity to p53 may identify a clinical subtype of AIH, we compared the clinical features of AIH patients with and without autoantibodies to p53. The serum levels of transaminase, γ-globulin and bilirubin were similar in the p53 seropositive group and in the p53 seronegative group (Table II); both groups responded equally well to immunosuppressive therapy. Moreover, there was no difference in the age at diagnosis or in the male to female ratio (Table II). All but one of the p53 seropositive AIH patients had at least one of the known AIH-susceptibility HLA alleles A1, B8, DR3, or DR4. There was no association of anti-p53 seropositivity with AIH subtypes according to the presence of autoantibodies [10 ANA, 11 smooth muscle antibody (SMA), 3 soluble liver antigen (SLA), 1 liver kidney microsomal (LKM)]. Three of the p53 seropositive patients had antibodies to double-stranded DNA (30%) and 7 had antibodies to single-stranded DNA (78%); in contrast, only 8 and 28% of the p53 seronegative patients had antibodies to double- or single-stranded DNA, respectively.

DISCUSSION

A significant proportion of AIH patients (31%) have elevated levels of antibodies to the p53 protein. It is unlikely, that this finding is due to a premalignant status or the presence of tumours, because the incidence of cancer in AIH is very low,[21,22] the titers of anti-p53 antibodies in tumour patients are usually higher, and the anti-p53 seropositivity in patients with AIH was correlated with the presence of antibodies to the p53 carboxy peptide (Fig. 2, Table I), a reactivity associated with autoimmune disease[14,20] rather than with tumour.[16] Autoimmunity to p53 in AIH patients may be related to p53 accumulation after inflammatory stress of the liver. However, though the livers of PBC and viral hepatitis patients are conceivably stressed as well, these patients did not manifest significant levels of p53 autoantibodies. The reason for the low prevalence of anti-p53 antibodies in the viral hepatitis group may be due to the interference of viral proteins with the p53 molecule;[23] the low prevalence of antibodies to p53 in the PBC group might be related to a lack of p53 involvement in the pathogenic process. Indeed, the tissue damage in PBC is mainly cholestatic, in contrast to the immune-mediated damage in AIH, and does not appear to be associated with p53 accumulation.[24] Autoantibodies to p53 may be a secondary marker of autoimmune inflammation and stress, though the different pathogenic molecular mechanisms in AIH and PBC need to be elucidated.

We have reported earlier that antibodies to DNA-binding domains such as that of p53 may be related to the generation of antibodies to DNA.[14,20,25] In the present

TABLE I Incidence of autoantibodies to p53 and the p53 carboxy-peptide

	Incidence of autoantibodies		Correlation of antibodies to p53 and to carboxy-peptide
	p53 Protein	p53 Carboxy peptide	r (95%, confidence interval)
Control ($n = 56$)	2 (4%)	2 (4%)	0.6926 (0.5114–0.8149)
AIH ($n = 45$)	14 (31%)	9 (20%)	0.8727 (0.7649–0.9330)
PBC ($n = 60$)	5 (8%)	4 (7%)	0.9162 (0.8482–0.9545)
Hepatitis B ($n = 22$)	1 (5%)	1 (5%)	0.8771 (0.7101–0.9507
Hepatitis C ($n = 55$)	4 (7%)	6 (11%)	0.5391 (0.3067–0.7108)

TABLE II Clinical features of anti-p53 seropositive and seronegative AIH patients

	p53 Seropositive	p53 Seronegative
AST (U/ml)	267 ± 107	265 ± 43 ($P = 0.2646$)
Bilirubin (mg/dl)	2.88 ± 1.93	2.78 ± 0.83 ($P = 0.2157$)
γ-globulin (%)	25.6 ± 2.5	26.3 ± 1.3 ($P = 0.4804$)
Age at diagnosis (years)	49 ± 17	46 ± 18 ($P = 0.6161$)
Ratio (female/male)	11:3	26:5 ($P = 0.6892$)
Cirrhosis at diagnosis	5 (35%)	12 (39%) ($P = 0.9998$)

study, we find antibodies to the carboxy-terminal DNA-binding domain of p53 in 20% of the AIH patients and 78% of the AIH patients with antibodies to the p53 carboxy-peptide also manifest antibodies to single- or double-stranded DNA. In contrast, only 28% of the AIH patients without antibodies to the p53 carboxy-peptide have antibodies to DNA. Thus, the presence of antibodies to p53 and notably to the carboxy-peptide may explain the relatively high prevalence of anti-DNA antibodies in AIH and their low prevalence in PBC.[26–29]

The presence of antibodies to p53 does not appear to characterise a clinical subtype of AIH and is not associated with the occurrence of ANA, SMA, LKM, or SLA. Nevertheless, the detection of antibodies to p53 may serve as an additional marker in the differential diagnosis of chronic hepatitis, since the prevalence of anti-p53 in AIH is higher than in other forms of chronic hepatitis.

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