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ALCOHOL DEHYDROGENASE: AN AUTOANTIBODY TARGET IN PATIENTS WITH ALCOHOLIC LIVER DISEASE

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The link between alcohol consumption and liver disease is not direct and several factors including autoimmunity to hepatocyte components have been implicated. We have previously identified alcohol dehydrogenase (ADH) as an autoantigen in autoimmune liver disease and in a proportion of patients with alcoholic liver disease. The aim of the present study is to investigate the association between the presence of anti-ADH antibodies, alcohol consumption and severity of liver damage in alcoholic patients. The presence of antibodies to human ADH β2 and horse ADH was investigated in 108 patients with documented history of alcohol consumption and alcohol related liver disease, 86 being active alcohol abusers and 22 on sustained alcohol withdrawal, 39 with non-alcohol related disease and 22 normal subjects.

Antibodies to either ADH form were more frequently detected in active alcohol abusers (55/86, 64%) than in patients on sustained alcohol withdrawal longer than 6 months (1/8, 13%, P<0.005), HBV infection (2/8, 25%, P=0.03), non-alcohol related disease (9/29, 23%, P<0.0001) and in normal controls (3/22, 14%, P<0.0001); were more frequent in patients with cirrhosis than in those with steatosis (26/34, 76% vs 34/64, 53%, P=0.02); and were associated with elevated levels of ALT (anti-ADH β2, P<0.05), immunoglobulin A (P<0.05) and γ-glutamyl transpeptidase (P=0.01). Anti-ADH antibody positive serum samples were able to inhibit the enzymatic activity of ADH. These findings suggest that anti-ADH antibodies may be triggered by alcohol consumption and act as a disease activity marker in alcoholic liver disease.

Excessive alcohol intake is associated with acute and chronic liver damage including steatosis, alcoholic hepatitis and cirrhosis (1-2). The connection between alcohol consumption and liver damage is not direct, however, since heavy drinking is not necessarily associated with hepatocyte damage and only one in six people who drink heavily develop cirrhosis (1). A number of contributory factors have been invoked to explain the link between excessive alcohol intake and liver disease (3-4), including genetic predisposition (5) and the triggering of an autoimmune attack on the hepatocyte (6).

Non-organ and liver specific autoantibodies have been described in patients with alcoholic liver disease (ALD), such as anti-liver specific lipoprotein (LSP) antibodies and their prevalence is associated with disease activity (7-10). Anti-LSP is more common in patients with active cirrhosis (60%) than in those with inactive cirrhosis or fatty changes (15%). An immunoregulatory defect was also shown in patients actively abusing alcohol but not when they stopped drinking (11). Alcohol *per se* may therefore induce alterations in the immune system predisposing to autoimmunity (7,12).

Keywords: autoimmunity, autoantibodies, alcohol consumption, isoenzymes, hepatitis C virus

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We have previously reported that human alcohol dehydrogenase (ADH) is a component of LSP and an autoantibody target in patients with autoimmune hepatitis (13). In the same study we noted that anti-ADH antibodies were present, at a prevalence similar to that seen in autoimmune liver disease (50%), in a small control group of patients with ALD and that observation triggered the present study (13).

Ethanol is metabolised mainly by the polymorphic liver ADH enzyme, the isoforms of which are variably composed of a (encoded by gene *ADH1*), β1, β2, β3 (*ADH2*), γ1, γ2 (*ADH3*) subunits sharing about 95% identity and closely related to each other in terms of their enzymatic activity, amino acid composition and immunological characteristics (14-15).

In the present study we have studied the presence of anti-ADH antibodies in a large series of patients both abusing alcohol and on alcohol withdrawal with documented liver disease and in patients without alcohol related liver or non-liver disease. We have investigated anti-ADH antibodies direct against human ADH β2, which is a recombinant protein purified through sequential chromatography, and horse ADH, which is highly homologous to human ADH and commercially available, and their dynamic changes during alcohol withdrawal. We also investigated whether anti-ADH antibody affects the catalytic activity of this enzyme.

MATERIALS AND METHODS

Patients and controls

One hundred and eight Italian patients with alcoholic liver disease were studied (95 males, median age 50 years, range 22 – 80). All patients were attending an out-patient clinic at the Department of Internal Medicine, University of Milan, Italy. The severity of cirrhosis was defined according to the Child criteria. The diagnosis and severity of alcoholic liver disease was based on a history of excessive alcohol consumption, clinical features of liver disease, biochemical findings, ultrasonography, esophagogastroscopy and liver biopsy (16). History of alcohol consumption was obtained through an interview and a Munchner Alcoholismusest questionnaire (17). Eighty-six patients were active alcohol abusers (AAA) defined on the basis of an alcohol intake exceeding 60 g/day in males and 40 g/day in females for more than five years (18). Nine of them were followed from the time of active alcohol abuse to that of alcohol withdrawal for a median of 40 days into abstinence (range 30 to 100 days). Twenty-two of the 108 patients were on sustained alcohol withdrawal (SAW), having abstained from alcohol intake 3 months (14 patients, SAW group A) to longer than 6 months (up to 3 years, 8 patients, SAW group B). As non-alcoholic controls, 39 hospital in-patients (25 males, median age 50 years, range 21 - 82) with non-alcohol related diseases were tested. They were in hospital for any reason other than surgery, anaemia, heart failure or diabetes. They had no history of alcohol intake. Twenty blood donors and two healthy laboratory workers (all male, median age 34 years, range 21 - 60) were studied as normal controls. The demographic and clinical data of patients and controls are presented in Table I. Aspartate aminotransferase (AST) and alanine transaminase (ALT) levels (upper normal limit: 45 IU/l), were assessed in all patients; g-glutamyl transpeptidase (γGT) levels (upper normal limit: 28 IU/l) and two markers of alcohol consumption: carbohydrate-deficient transferrin (CDT) (upper normal limit: 20 U/l for man and 26 U/l for woman) and mean corpuscular volume (MCV, upper normal limit: 90fl) were assessed in 106 ALD patients and in all pathological control patients. CDT levels were determined with a radioimmunoassay (CD-TectTM, Kabi Pharmacia). Immunoglobulin A (IgA) levels (normal value <4.0 g/l) were measured in 89 ALD patients using a Behring Nephelometric Analyzer II and nephelometric grade specific antisera (Behring Diagnostics Inc., San José, CA, USA). Patients were also divided into groups based on whether they had steatosis, hepatitis or cirrhosis (Table II). This study was approved by the local Ethical Committee.

Presence of HBsAg, anti-HBs, HBeAg, anti-HBe and antibodies to hepatitis B core antigen was tested by commercial enzyme-immunoassays (Abbott Laboratories, North Chicago, IL, USA). Antibodies to hepatitis C virus were detected by ELISA II (Ortho Diagnostics System, Raritan, NJ, USA) and confirmed by RIBA II (Ortho Diagnostics System and Chiron Corp, Emeryville, CA, USA) according to the instructions of the manufacturer.

Detection of Non-organ specific autoantibodies

Anti-nuclear (ANA), anti-mitochondrial (AMA), anti-smooth muscle (SMA) antibodies and liver kidney microsomal antibody type 1 (LKM1) were investigated by indirect immunofluorescence on 5-mm cryostat sections of rat liver, kidney and stomach (19) at the

initial dilution of 1/10 in phosphate buffer saline (PBS). Positive sera were double-diluted to extinction.

Preparation of human ADH β2

The preparation of recombinant human ADH β2 isoform has been described in detail elsewhere (20-21). In brief, the cDNAs of ADH β2 was obtained from a human liver cDNA library using appropriate synthetic nucleotide probe and was ligated into plasmid pKK223-3. The ligation mixtures were then transformed into *E.coli* JM105. Purified recombinant ADH β2 was obtained from the lysate supernatant by sequential chromatography.

Detection of anti-ADH antibodies by immunoblot

Antibodies to human ADH β2 subunit and horse liver ADH (17233, Sigma Aldrich, Poole, UK) were detected by immunoblot (13). In brief, ADH preparations were subjected to electrophoresis in 12% polyacrylamide mini gels (Bio-Rad Laboratories, Hemel Hempstead, UK) and then transferred onto nitrocellulose filters. Non-specific binding was blocked with 1% gelatin. Filter strips were incubated with patients' sera at an initial dilution of 1/300, and polypeptides targeted by anti-ADH antibodies were initially visualised using peroxidase-conjugated rabbit anti-human IgA, G and M (DAKO, Copenhagen, Denmark) and thereafter using anti-human IgG (Dako) at a dilution of 1/750 for 1 h at room temperature. Positive sera were diluted further at 1/600, 1/1000, 1/2000 and 1/3000.

Measurement and inhibition of horse ADH enzymatic activity

Enzyme activity of ADH was measured by the production of NADH during incubation of ethanol (33 mM) and NAD^+ (2.4 mM) in glycine buffer (0.8ml, 100mM, pH 10) for 200 minutes, with or without the presence of serum (5μl) from subjects either anti-ADH antibody positive or negative, or ADH alone. NADH concentration was calculated using an extinction coefficient of $6250 M^{-1}cm^{-1}$ at 340nm, measured using an Agilent 8453 diode array spectrophotometer (Agilent, UK) (22).

Statistical analysis

Anti-ADH antibody prevalence in the different groups was compared using the χ^2 test. The normality of variable distributions was tested using the Kolmogorov-Smirnov goodness of fit test and found that they were not normally distributed. Therefore, differences in levels of AST, ALT, CDT, MCV, γGT and IgA between groups were analyzed by Wilcoxon's rank sum test. P values of <0.05 are considered significant.

RESULTS

Prevalence of anti-ADH antibodies in patients with ALD and its association with severity of liver disease

Amongst undivided ALD patients, antibodies to human ADH β2 and horse ADH were present in 58 (54%) and 48 (44%) of the patients respectively; the prevalence of antibodies to either ADH was 61% (66/108) (Table II). Reactivities to human ADH β2 and horse ADH were closely related (χ^2 value=35, P<0.0001). Fig. 1 shows the presence of antibodies to human ADH β2 in the sera of representative patients with ALD and in one normal subject. Anti-human ADH β2 antibodies were detected more frequently in patients with cirrhosis (65%) than in those with steatosis (44%, P<0.05). The highest prevalence of anti-ADH β2 was seen in patients with alcoholic hepatitis (75%) (Table II).

Associations between anti-ADH antibodies and biochemical markers of alcoholic liver disease

The associations between anti-ADH antibodies and biochemical markers of alcoholic liver disease are summarized in Table III. Prevalence of antibodies to ADH β2, horse ADH or either ADH was higher in patients with elevated γGT levels than in those with normal levels (P=0.003, P<0.05 and P=0.01 for anti-ADH β2, –horse ADH and –either-ADH antibody respectively). Similarly, the prevalence of anti-ADH β2 was higher in patients with elevated ALT (77%) than in those with normal ALT levels (48%, P<0.05) and the prevalence of antibodies to ADH β2 was higher in patients with elevated IgA levels (P=0.016 for anti-ADH β2 and P<0.05 for anti either ADH). Conversely, patients positive for anti-ADH β2 had higher IgA levels (median 4.16 g/l) than those negative (median 2.89 g/l, P=0.006). Frequency of anti-ADH antibodies tended to be higher in patients with elevated CDT and MCV levels (Table III).

Association between anti-ADH antibodies and alcohol consumption

Associations between anti-ADHs antibodies

Table I. *Demographic, biochemical, clinical, immunological data in patients with alcoholic liver disease, non-alcoholic and normal controls.*

Subjects	Age Yrs Range (median)	Sex M/F	CDT U/l	MCV U/l	AST IU/l	ALT IU/l	γGT U/l	IgA g/l	Liver disease number (%) Steatosis	Hepatitis/ cirrhosis	Cirrhosis	Positive for conventional AAb number (%)	Anti-HCV Ab Number of Positive/tested (%)
Alcoholic liver disease n=108	22-80 (50)	95/13	4.2-54.6 (20.5)	79-122 (97)	5-155 (19)	4-149 (16)	6-1160 (79)	0.75-9.4 (3.5)	64 (59)	8 (6)	34 (31)	15 (14)	18/92 (20)
AAA, drinking alcohol >60g/day, n=86	22-80 (50)	78/8	4.2-54.6 (21.2)	79-122 (98)	5-155 (23)	4-149 (18)	11-1160 (90)	0.75-9.4 (3.6)	58 (67)	4 (5)	24 (28)	12 (14)	16/80 (20)
SAW group A no drinking for 3 months n=14	25-72 (54.5)	10/4	9.4-42 (16.7)	82-111 (95.5)	7-63 (15.5)	6-99 (13.5)	8-78 (30.5)	nd	0	4 (29)	10 (71)	3 (21)	2/8 (25)
SAW group B no drinking for 6 months n=8	47-73 (49)	7/1	3.6-23.7 (16.95)	87-102 (92)	5-15 (11)	6-10 (8)	6-57 (7)	1.32-5.29 (2.92)	6 (75)	0	0	0	0/4 (0)
Non-alcoholic controls n=39 Hospital-in patients, no drinking history	21-82 (46)	16/23	6-25 (11.1)	63-96 (86)	5-21 (7.5)	5-34 (8)	3-51 (11)	nd	0	0	0	1 (3)	1/25 (4)
Normal controls n=22	21-60 (34)	22/0	nd	nd	nd	nd	nd	nd	0	0	0	0	nd

AAA, active alcohol abusers; ALT, alanine transaminate, AST, aspartate aminotransferase; conventional AAb, autoantibodies including anti-nuclear, -smooth muscle and -liver kidney microsomal antibodies; CDT, carbohydrate-deficient transferring; F, female; HCV, hepatitis C virus; M, male; MCV, mean corpuscular volume; SAW, subjects on sustained alcohol withdrawal; nd, not done; Yrs, years.

Table II. *Association between anti-ADH antibodies and documented severity of liver damage in patients with alcoholic liver disease.*

Disease category	Prevalence of anti-ADH antibodies, number of positive (%) Human ADH β2	P value*	Horse ADH	P value*	To either ADH form antibody	P value*
Undivided patients, n=108	58 (54)		48 (44)		66 (61)	
Steatosis, n=64	28 (44)		26 (41)		34 (53)	
Alcoholic hepatitis, n=8	6 (75)	0.09	3 (38)	NS	6 (75)	NS
Cirrhosis, n=34	22 (65)	**<0.05**	19 (56)	NS	26 (76)	**0.02**
Child A, n=10	7 (70)	NS	5 (50)	NS	8 (80)	NS
Child B, n=17	12 (71)	**<0.05**	11 (65)	0.07	14 (82)	**0.03**
Child C, n=7	3 (43)	NS	3 (43)	NS	4 (57)	NS
Unknown, n=2	0 (0)		0 (0)		0 (0)	

** P values refer to comparisons of anti-ADH antibody prevalence in patients with steatosis to those with alcoholic hepatitis and cirrhosis. NS, not significant.*

Table III. *Association between anti-ADH antibodies and the biochemical markers of alcoholic liver disease.*

Biochemical markers n=number of cases tested	Prevalence of anti-ADH antibodies, number of positive (%)					
	Human ADH β2	P value*	Horse ADH	P value*	To either ADH form antibody	P value*
γGT, n=106						
abnormal, n=81	50 (62)	**0.003**	41 (51)	**<0.05**	55 (68)	**0.01**
normal, n=25	7 (28)		7 (28)		10 (40)	
AST, n=108						
abnormal, n=23	12 (52)	NS	11 (48)	NS	15 (65)	NS
normal, n=85	43 (51)		37 (44)		51 (60)	
ALT, n=108						
abnormal, n=13	10 (77)	**<0.05**	6 (46)	NS	10 (77)	NS
normal, n=95	46 (48)		42 (44)		56 (59)	
IgA, n=89						
abnormal, n=38	24 (63)	**0.016**	18 (47)	NS	26 (68)	**<0.05**
normal, n=51	19 (37)		21 (41)		24 (47)	
CDT, n=106						
abnormal, n=52	32 (62)	NS	25 (48)	NS	35 (67)	NS
normal, n=54	25 (46)		24 (44)		30 (56)	
MCV, n=106						
abnormal, n=52	30 (58)	NS	25 (48)	NS	34 (65)	NS
normal, n=54	27 (50)		25 (46)		32 (59)	

** P values refer to comparisons of anti-ADH antibody prevalence in patients with abnormal biochemical markers to those with normal values. ADH, alcohol dehydrogenase. CDT, carbohydrate-deficient transferring; MCV, mean corpuscular volume; NS, not significant.*

Table IV. *Prevalence of anti-ADH antibodies in alcoholics, non-alcoholic and normal controls.*

Subjects	Prevalence of anti-ADH antibodies, number of positive (%)					
	Human ADH β2	P value*	Horse ADH	P value*	Either anti-ADH antibodies	P value*
Alcoholics, n=108	58 (54)		48 (44)		66 (61)	
AAA, n=86	48 (56)		41 (48)		55 (64)	
SAW A, no drinking for 3 months n=14	9 (64)	NS	7 (50)	NS	10 (71)	NS
SAW B, no drinking for 6 months n=8	1 (13)	**0.02**	0	**0.01**	1 (13)	**<0.005**
Non-alcoholic controls, n=39	7 (18)	**<0.0001**	4 (10)	**<0.0001**	9 (23)	**<0.0001**
Normal controls, n=22	2 (9)	**<0.0001**	2 (9)	**0.001**	3 (14)	**<0.0001**

** P values refer to comparisons of anti-ADH antibody prevalence in AAA to those in other groups. ADH, alcohol dehydrogenase; AAA, active alcohol abusers; SAW, sustained alcohol withdrawal; NS, not significant.*

Table V. *Demographic, clinical and immunological data in 5 alcoholics at the time of alcohol abuse and on abstinence.*

Patients	Age (yrs)	Sex	Abstinence (days)	Liver disease	Anti-ADH antibodies human ADH β2		horse ADH		CDT U/l		MCV* U/l		AST IU/l		ALT IU/l		γGT* U/l		IgA# g/l		Anti-HCV Antibodies	
					number of positive (%)				*Median*													
					4 (44)	1 (11)	5 (55)	2 (22)	*23*	*18*	*105*	*102*	*21*	*26*	*18*	*17*	*61*	*38*	*5.11*	*3.63*		
					1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd	1st	2nd
No 1	62	M	45	steatosis	neg	neg	1/300	neg	25.7	17.5	106	103	12	6	18	5	25	7	2.99	1.20	neg	neg
No 14	50	M	40	steatosis	1/1000	1/1000	1/3000	1/1000	51.6	23.6	103	97	21	26	19	15	41	38	2.78	3.04	neg	neg
No 15	52	M	40	steatosis	1/300	neg	1/300	neg	34.8	57.0	101	96	16	34	12	17	31	37	4.16	3.63	neg	neg
No 35	62	M	90	cirrhosis	1/300	neg	1/300	neg	23.0	22.4	103	96	13	13	6	7	61	18	7.20	5.07	ND	neg
No 43	45	M	100	cirrhosis	1/300	neg	neg	neg	25.4	19.5	106	103	31	59	13	20	184	250	8.82	7.57	neg	ND

1st, measurements when patients actively abusing alcohol; 2nd, measurements when patients remained in abstinence; ADH, alcohol dehydrogenase; ALT, alanine transaminate, AST, aspartate aminotransferase; AAb, autoantibodies; CDT; carbohydrate-deficient transferring; HCV, hepatitis C virus; M, male; MCV, mean corpuscular volume; neg, negative; ND, not done; NS, not significant.

**: The first value was significantly higher than the second, P=0.01 for MCV and P=0.03 for γGT respectively.*

#: The first value tended to be higher than the second, P=0.08.

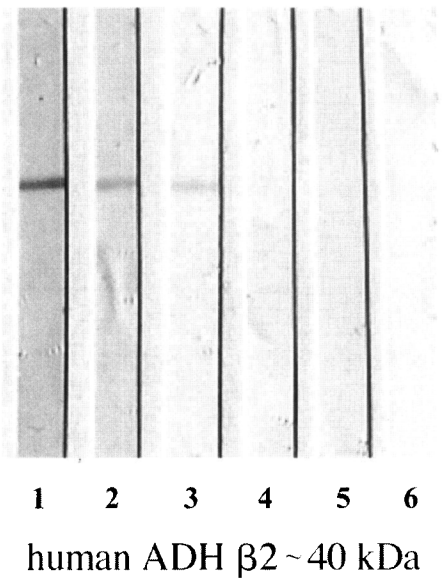

Fig. 1. *Immunoblot analysis of serum reactivity against human ADH β2. ADH β2 was subjected to electrophoresis in 12% polyacrylamide mini gels and sera were applied at 1/300 dilution. Lanes 1–3, anti-ADH antibody positive serum samples from three representative cases with alcoholic liver disease (ALD) and active consumption of alcohol. A band of variable intensity is present at ~40 kDa corresponding to human ADH β2; lanes 4–5, sera from two anti-ADH antibody negative patients with ALD that have stopped drinking alcohol for longer than 6 months; lane 6, a healthy subject unreactive to human ADH β2.*

and drinking patterns are given in Table IV. Antibodies to human ADH β2 were present in 56% AAA patients, the frequency being higher than in SAW group B (13%, P=0.02), non-alcoholic patients (18%, P<0.0001) and normal controls (9%, P<0.0001), but was similarly present in those in SAW group A (in abstinence for three months). Antibodies to horse ADH were also more prevalent in AAA patients compared to the SAW group B, non-alcoholic patients and healthy subjects. Moreover, reactivity to at least one form of ADH was more frequent in AAA than in SAW group B (P=0.0046), HBV infection (P=0.03), non-alcoholic patients (P<0.0001) and healthy subjects (P<0.0001).

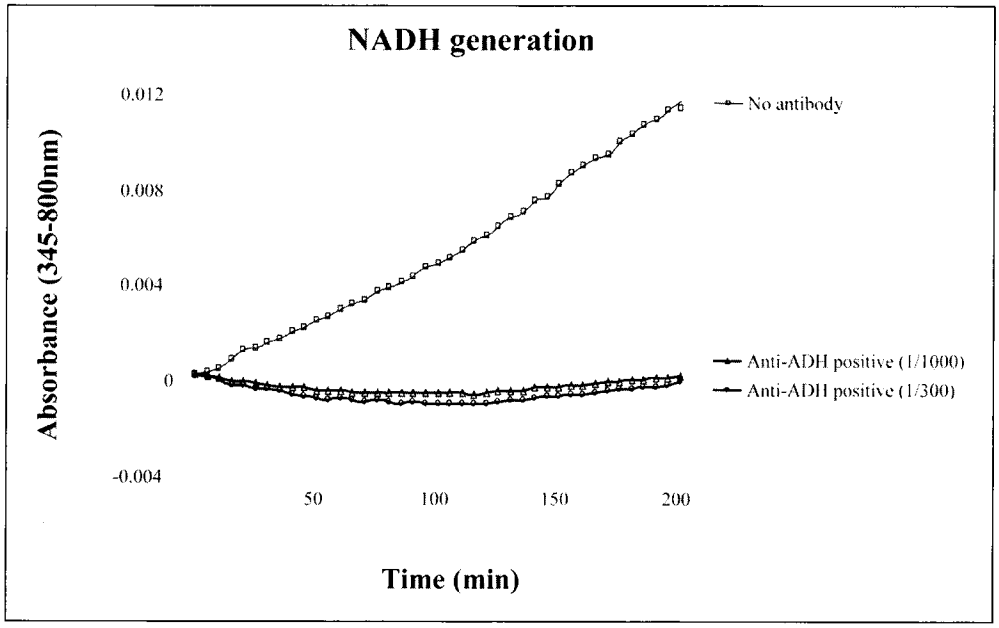

Fig. 2. *Inhibition of ADH enzymatic activity by sera positive for anti-ADH antibodies. ADH enzymatic activity was measured by the production of NADH during incubation of ethanol and NAD^+ at the presence of horse ADH and sera from two representative patients positive for anti-horse ADH antibody (1/300 and 1/1000 respectively). Y axis indicates the production of NADH and X axis the duration of investigation in minutes. Anti-ADH antibody completely inhibits activity of enzyme over the time period of the assay (the second and third lines).*

Nine AAA patients were followed when they were into abstinence for a median of 40 days (range 30 to 100 days). Levels of MCV, γGT and IgA were lower during abstinence than at the time of drinking. In five AAA positive for antibodies to either form of ADH and studied during abstinence, the antibody levels decreased or became negative in 4 at days 40, 45, 90 and 100 into abstinence. Their demographic, clinical and immunological data are summarized in Table V.

Non-organ specific autoantibodies in patients with AAA

Non-organ specific autoantibodies were present in 12 of the 86 (14%) AAA patients: 8 had anti-smooth muscle antibodies (SMA) (titre range 1/20 to 1/80, median 1/40), 2 anti-nuclear antibodies (ANA) (titre 1/20), 1 anti-mitochondrial antibody (AMA) (titre 1/20) and 1 anti-liver kidney microsomal antibody type 1 (LKM1) (titre 1/20). Three SAW patients (14%) were positive for non-organ specific antibodies: 1 had ANA (titre 1/160), 1 SMA (titre 1/80) and 1 LKM1 (titre1/160).

Inhibition of ADH enzymatic activity by sera positive for anti-ADH antibodies

Inhibition of ADH activity was investigated in 7 ALD patients, four being positive for anti-ADH β2 and horse ADH (titre 1/300 to 1/1000) and three negative and in two normal controls. NADH generation was found to be linear with respect to time in tubes containing horse ADH only, sera from normal controls and ALD patients negative for anti ADH antibody. However, there was a lag phase in the time course up to 200 minutes of NADH production in the tubes containing sera from three ALD patients who were positive for anti-ADH antibodies (titre 1/300 to 1/1000) (Fig. 2).

DISCUSSION

Our results show that approximately two thirds of patients actively abusing alcohol produce autoantibodies against alcohol dehydrogenase, the enzyme central to ethanol oxidation, with 64% of them being anti-ADH autoantibody positive

compared to only 13% of patients on sustained alcohol withdrawal.

There is evidence that alcohol ingestion *per se* may be sufficient to trigger the production of autoantibodies (7). Intragastrically alcohol fed rats produce autoantibodies to cytochrome P450 2E1 (CYP2E1), a major enzyme of the ethanol oxidising system, before the appearance of liver disease (23). In our own study, anti-ADH antibody prevalence was significantly lower in patients who had stopped drinking for more than 6 months than in those who did not stop drinking, indicating that alcohol withdrawal is accompanied by the disappearance of the autoantibody. This view was reinforced by the finding that three of four anti-ADH positive patients studied prospectively became anti-ADH seronegative after stopping drinking for a period ranging from 40 to 100 days. That these patients had effectively stopped drinking is indicated by the decrease in indices of the alcohol abuse such as MCV, γGT and IgA. Taken together, these data suggest that alcohol may indeed trigger anti-ADH autoantibody production directly.

The observation that anti-ADH antibodies were frequent in active alcohol abusers and closely related to alcohol consumption while non-organ specific antibodies such as ANA, SMA and LKM were relatively uncommon in the same patients, with a similar prevalence in alcohol abusers and in those on sustained alcohol withdrawal, further strengthens the link between abuse of alcohol and production of autoantibodies to the alcohol-metabolising ADH. Within the group of alcohol abusers, anti-ADH antibodies were more prevalent in patients with cirrhosis or with elevated levels of ALT than in those with steatosis or with normal levels of ALT. It is possible that a more severe liver disease and a higher prevalence of anti-ADH antibodies both reflect a higher alcohol intake. It is also possible that the ADH released from the cytoplasm of damaged hepatocytes acts as a stimulus to continuous autoantibody production. Relevantly, the highest prevalence of anti-ADH antibodies was found in patients with alcoholic hepatitis, a condition characterized by high levels of cytosolic liver enzymes circulating (7).

Whether the humoral immune reaction to a key enzyme in ethanol metabolism such as ADH also contributes to the pathogenesis of alcoholic liver disease or is just a consequence of alcohol-induced liver damage remains to be established (13). The fact that ADH autoantibodies can be found in some 10% of the normal controls would suggest that they are not influential in the development of alcoholic liver disease. On the other hand, the finding presented here that anti-ADH antibodies inhibit the activity of the native enzyme appears to indicate their pathogenic role, especially if the enzymatic inhibition observed *in vitro* also operates *in vivo* (24). Antibodies to ADH delay NADH production, raising the possibility of a metabolic defect (Fig. 2). How this translates to an *in vivo* effect is still unclear. Prospective studies, investigating whether alcohol abusers with anti-ADH antibodies are at a greater risk of liver damage than those anti-ADH antibody negative, are warranted and will give us an insight as to the pathogenic significance of our observations and their relevance to hepatocyte destruction. An autoantibody induced inhibition of the ADH catalytic activity would favour ethanol metabolization through the CYP2E1 pathway or by gut bacteria (12); the free radicals generated in the first instance and endotoxin produced in the second are both capable of hepatocyte damage (12). Against a pathogenic role for anti-ADH antibodies is the fact that their target is a cytosolic enzyme and, according to classical views, inaccessible to the effector molecules of the immune system. This notion has been challenged, however, by studies showing that antibodies directed to intracellular components, such as nuclear antigens, are capable of inflicting damage *in vivo* (25-28). Of further relevance in understanding the pathogenic process is the fact that anti-ADH antibodies belong to the IgG isotype, implicating T-cell 'help' in their generation (29-32). This raises the possibility that ADH tolerance breakdown occurs not only at B cell level, but also T cell level, a possibility requiring future study.

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