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Increased Serum Concentration of IgA2 Subclass and IgA2/IgA1 Ratio: Specific Markers of Chronic Alcoholic Abuse?

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Summary: Enhanced serum IgA concentrations are common in alcoholic liver cirrhosis, but functional differences between IgA subclasses and their relation with interleukin-6 (IL-6) have not been described. Distinct immunoregulatory mechanisms may exist that selectively affect one subclass. This possibility prompted us to investigate the distribution of IgA1 and IgA2 subclasses in the serum of 25 heavy alcohol drinkers (alcohol: 80 to 200 g per day) without clinical disorders, in comparison with 35 patients affected by alcoholic liver cirrhosis, 29 viral hepatitis patients and 33 social drinkers as a control group. Mean (± SD) IgA2 concentration (0.56 ± 0.31 g/l) was significantly increased ($p < 0.01$) in heavy alcohol drinkers, with an IgA2/IgA1 ratio of 0.33 ± 0.12, while the mean total IgA concentration was similar to the control group. Mean IgA1 and IgA2 concentrations were significantly increased ($p < 0.001$) in alcoholic liver cirrhosis patients (6.13 ± 4.52 g/l and 1.83 ± 1.93 g/l respectively, with an IgA2/IgA1 ratio of 0.32 ± 0.19) and viral hepatitis patients (3.66 ± 2.59 g/l and 0.69 ± 0.67 g/l respectively, with an IgA2/IgA1 ratio of 0.21 ± 0.14) High serum IL-6 concentrations (34 ± 33 ng/l) were correlated with elevated IgA1 and IgA2 concentrations only in patients with alcoholic liver cirrhosis. IgA2 subclass and IgA2/IgA1 ratio could therefore be used as markers of chronic alcohol abuse directly related to the extent and duration of the alcohol abuse and the effectiveness of alcohol withdrawal.

Introduction

Abnormalities in the immune systems of alcoholic liver cirrhosis patients have been described (1–4). However, their pathogenic significance and clinical implications remain unknown. Hypergammaglobulinaemia, mainly enhancement of the serum IgA concentration, is a common immunological finding in patients with alcoholic liver cirrhosis (5–7). It has been demonstrated that this high immunoglobulin concentration is not due to abnormal catabolism of IgA (1–2).

Swerdlow et al. have shown in patients with alcoholic liver disease that the IgA2 subclass forms a major subclass component contributing to the continuous pattern of IgA deposition in hepatic tissues (8).

Recently, high serum IgA2 concentrations were found in heavy alcohol drinkers without alcoholic liver cirrhosis (9). This subclass may be a predictive marker for evolution to alcoholic liver disease.

In addition, increased serum concentrations and in vitro spontaneous or induced production of interleukin-6 (IL-6) peripheral blood monoclonal cells have been reported in patients with alcoholic liver cirrhosis (10). IL-6 production correlates closely with IgA serum levels. This abnormality may be related to overproduction of IgA and immune disturbances in patients with alcoholic liver disease.

We have studied serum IgA, IgA1, IgA2 and IL-6 concentrations in heavy alcohol drinkers without clinical liver disease in comparison with alcoholic liver cirrhosis and viral hepatitis patients to clarify the significance of the high serum IgA2 concentration in relation to alcohol consumption.

Patients and Methods

Patients

The biochemical hepatic characteristics of the four groups are given in table 1.

– The control group comprised thirty-three healthy subjects (12 women and 21 men, mean age ± SD: 42 ± 12 years, range: 24–56 years) who were social drinkers (alcohol ingestion < 40 g per day) with normal hepatic characteristics.

Tab. 1 Hepatic characteristics (mean ± SD (median)) of the studied population.

Group	n	Alanine amino-transferase (U/l)	Aspartate amino-transferase (U/l)	γ-Glutamyl-transferase (U/l)
Reference values (30 °C)		4 − 45	10 − 45	5 − 40
Control social drinkers	33	17 ± 16 (15)	18 ± 15 (13)	21 ± 20 (18)
Heavy alcohol drinkers	25	25 ± 5 (23)	20 ± 5 (21)	55** ± 8 (54)
Alcoholic cirrhosis	35	67* ± 80 (55)	157** ± 75 (139)	534** ± 523 (248)
Viral hepatitis	29	106** ± 57 (111)	85* ± 69 (75)	71** ± 28 (70)

* $p < 0.01$; ** $p < 0.001$ vs control social drinkers (U-test of *Mann & Whitney*).

− Twenty-five heavy alcohol drinkers (10 women and 15 men, mean age ± SD: 52 ± 10 years, range: 35−70 years) who had ingested more than 80 g alcohol daily for at least five consecutive years without clinical or biological signs of liver disease were volunteers for a course of alcohol withdrawal.

− Thirty-five patients (7 woman and 28 men, mean age ± SD: 48 ± 11 years, range: 31−74 years) with alcoholic liver cirrhosis were grouped according to the *Child-Pugh* classification (11). They were studied at least two months after cessation of alcohol intake. In all cases, cirrhosis was due to chronic alcohol ingestion and confirmed by histology. None of the patients had overt signs of viral hepatic infection.

− Twenty-nine patients (12 women and 17 men, mean age ± SD: 50 ± 15 years, range: 17−71 years) with viral hepatitis B (n = 13), C (n = 11) and non-A, non-B, non-C (n = 5) were controlled in this study. The hepatitis was diagnosed according to conventional criteria, including serological markers using currently available assays and liver biochemical test abnormalities (alanine amino-transferase > 1.5 the upper normal limit for longer than six months). None of the viral hepatitis patients had liver cirrhosis.

In alcoholic liver cirrhosis and viral hepatitis patients, alcohol ingestion was less than 40 g per day at the time of the sample.

All patients gave their informed consent to the experimental protocol.

Serum samples

Blood samples were collected in sterile, clean, dry tubes and rapidly separated after coagulation. Serum was stored at −20 °C.

Measurement of serum IgA, IgA1 and IgA2 concentrations

Concentrations of IgA, and IgA1, IgA2 subclasses in serum were measured by a sandwich time-resolved immunofluorometric assay as previously described (12−14). The plates for microtitration (Microwell-Maxisorp™, Nunc, Roskilde, Denmark) were coated overnight at 4 °C with 200 µl of polyclonal anti-human α-chain (Dako, Glostrup, Denmark), at 0.005 g/l for IgA and IgA1, and at 0.010 g/l for IgA2, diluted in 0.05 mol/l K_2HPO_4 buffer adjusted to pH 8.5. Excess binding sites were blocked by three washes with 0.05 mol/l NaH_2PO_4 di-hydrate containing 5 g/l bovine serum albumin and 60 g/l sorbitol. Six serial dilutions of the OSAU standard (Behring, Marburg, Germany; IgA = 2.45 g/l, IgA1 = 2.02 g/l and IgA2 = 0.43 g/l) and two dilutions of the tested samples, in 0.1 mol/l Tris-HCl buffer adjusted to pH 7.75 (added to 0.15 mol/l NaCl, 5 g/l bovine serum albumin, 1 g/l Tween 20 and 0.02 mol/l di-ethylene-triamino pentacetic acid), were applied in duplicate and incubated for 2 hours at 20 °C with continuous shaking. After three washes with 0.05 mol/l Tris-HCl buffer at pH 7.75 containing 0.25 g/l Tween 20, 200 µl of the following europium-labelled antibodies (0.00025 g/l) were added for 1 hour at 2 °C with gentle shaking: anti-human α-chain (Dako), monoclonal IgG1K to human α1-chain (clone NI 69-11, Nordimmune™, Nordic, The Netherlands) and monoclonal IgG1K to human α_2-chain (clone NI J 12, Nordimmune™, Nordic). Two hundred µl of the enhancement solution (Wallac, Turku, Finland) were then added for 10 minutes with continuous shaking to dissociate the europium ions from the labelled immune complexes, providing highly fluorescent chelates (15). The fluorescence level was measured after a rest period of 10 minutes, using a fluorometer with a xenon flash lamp (1230 Arcus™ Fluorometer, Wallac). The concentrations of IgA, IgA1 and IgA2 were calculated by the standard curves.

The reliability of the reagents used in this assay had been previously verified (specificity 99% for anti-IgA1 and 98% for anti-IgA2; sensitivity 1 µg/l for both subclasses; linearity 10−1000 µg/l for IgA1 and 10−400 µg/l for IgA2; intra-assay variations 4.7−6.1%; inter-assay variations 6.7−9.1%) (12).

Measurement of serum interleukin-6 concentration

Level of IL-6 in serum was measured by means of an enzyme amplified sensitivity immunoassay, performed on a microtitration plate (Medgenix, Fleurus, Belgium). It is based on the oligoclonal system in which several monoclonal antibodies directed against distinct epitopes of IL-6 are used. The assay was performed directly on serum without any treatment or extraction. The amount of substrate (tetramethylbenzidine-H_2O_2) turnover was determined colorimetrically by measuring absorbance at 450 mm.

Statistical analysis

Results were expressed as arithmetic mean ± standard deviation (SD) and median. For the different properties, results were expressed as frequency (%) in comparison with the normal upper limit (mean ± 2 SD) of each property obtained for the control social drinkers. The *Mann & Whitney* U-test was used to compare the data from each group versus the control group. For correlation studies, *Spearman*'s rank correlation was used. Values of $p \leq 0.05$ were taken as significant.

Results

The results of the measurement of IgA, IgA1 and IgA2 subclasses and IL-6 concentrations are expressed in table 2 and IgA2/IgA1 ratios in figure 1.

Serum IgA, IgA1 and IgA2 concentrations and IgA2/IgA1 ratio

In this study, there was no difference in IgA subclass concentrations and IgA2/IgA1 ratios between men and women in the heavy alcohol drinkers and alcohol liver cirrhosis patients, even though women are far more prone to alcohol-induced liver damage.

− In the heavy alcohol drinkers, the IgA concentration was elevated in 3/25 (12%) of patients; the prevalence of elevated concentrations of IgA1, 1/25 (4%) and IgA2, 7/25 (28%) differed significantly

Tab. 2 Serum concentration (mean ± SD (median)) of the IgA, IgA1, IgA1 and interleukin-6.

Group	n	IgA (g/l)	IgA1 (g/l)	IgA2 (g/l)	IL-6 (ng/l)
Control social drinkers	33	2.42 ± 0.86 (2.31)	2.18 ± 0.73 (2.27)	0.39 ± 0.34 (0.28)	3.8 ± 1.9 (3)
Heavy alcohol drinkers	25	2.30 ± 1.30 (2.12)	1.77* ± 1.03 (1.56)	0.56** ± 0.31 (0.55)	4 ± 1.9 (3)
Alcoholic cirrhosis	35	7.61** ± 5.29 (7.01)	6.13** ± 4.52 (4.34)	1.83*** ± 1.93 (1.20)	34*** ± 33 (22)
Viral hepatitis	29	4.45** ± 3.03 (3.47)	3.66* ± 2.59 (2.60)	0.69* ± 0.67 (0.45)	150* ± 322 (12)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ vs control social drinkers (U-test of *Mann & Whitney*).

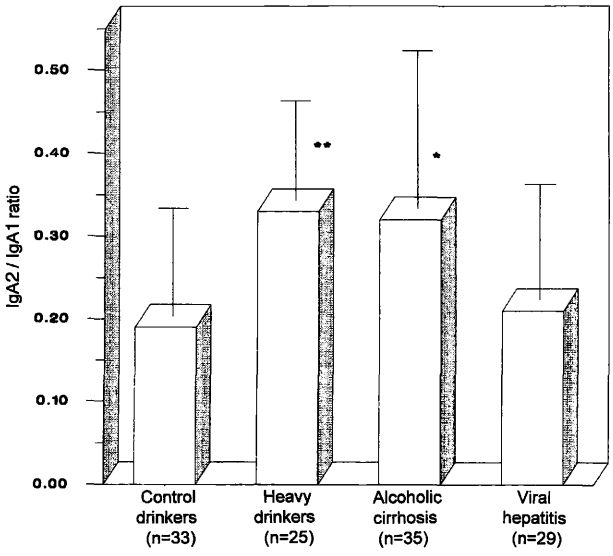

Fig. 1 IgA2/IgA1 ratio (mean ± SD) in control drinkers, in heavy drinkers, and in patients with liver disease.
* $p < 0.01$; ** $p < 0.001$ vs control drinkers (U-test of *Mann & Whitney*).

($p < 0.01$), with a predominance of IgA2. The IgA2/IgA1 ratio was elevated in 8/25 of patients (32%).

- In alcohol liver cirrhosis patients, the IgA concentration was elevated in 22/35 patients (63%); the prevalence of the elevated concentrations did not differ between IgA1, 20/35 (57%) and IgA2 22/35 (63%). The IgA2/IgA1 ratio was elevated in 9/35 patients (26%).
- In viral hepatitis patients, the IgA concentration was elevated in 12/29 patients (41%), the prevalence of elevated concentrations of IgA1, 11/29 (38%) and IgA2 5/29 (17%) differed significantly ($p < 0.01$), with a predominance of IgA1. The IgA2/IgA1 ratio was elevated in 3/29 patients (10%).

Serum concentration of interleukin-6

Mean IL-6 concentration was significantly increased in patients with alcohol liver cirrhosis ($p < 0.001$) and in viral hepatitis patients ($p < 0.05$). In this last group, there was a wide range of values (3−1230 ng/l). In these two groups, the prevalence of elevated concentrations of IL-6 was 10/17 (59%) and 12/23 (52%) respectively, and did not significantly differ.

Correlations between serum IgA, IgA1 and IgA2, and interleukin-6 concentrations

In the heavy alcohol drinkers, IgA1 and IgA2 concentrations were positively correlated ($p < 0.01$). In contrast, in alcohol liver cirrhosis and viral hepatitis patients, the elevated IgA1 and IgA2 serum concentrations were inversely correlated. In patients with alcoholic liver cirrhosis, a significant positive correlation ($p < 0.01$) was observed between elevated IgA1 and IgA2 concentrations and IL-6 secretion.

Discussion

The reliability criteria for the time-resolved immunofluorometric assay used for the measurement of total IgA and IgA1, and IgA2 subclasses were very satisfactory. This non-isotopic method is simple to perform and its high sensitivity makes it suitable for the determination of serum IgA1 and IgA2 concentrations in patients with alcoholic liver disease.

Heavy alcohol drinkers and alcoholic cirrhosis patients

In the heavy alcohol drinkers and the alcoholic liver cirrhosis patients, in whom the alcohol factor was present, the study showed an increased IgA2/IgA1 ratio. Whatever the total IgA concentration, the ratios in the heavy drinkers and alcoholic cirrhosis patients (0.33 ± 0.12 and 0.32 ± 0.19, respectively) were significantly different from the control social drinkers and the viral hepatitis patients. This agrees with our previous studies in which serum IgA subclass distributions were anlyzed in alcohol liver disease (9). The increase in IgA2/IgA1 ratio could constitute a diagnostic or predictive marker in heavy alcohol drinkers (prevalence: 32%) evolving to cirrhosis (26%), while this prevalence in viral hepatitis patients is 10%. In heavy alcoholic drinkers without clinical liver disease, microvascular changes in the intestinal tract may be considered as the cause of the plasma protein loss into the jejunal lumen demonstrated by *Buell & Beck*, and *Ray* et al. in the dog (16, 17). Several studies have shown the direct toxic effects of alcohol on intestinal epithelial cells and hepatocytes (18, 19). In particular, loss of superficial cells and damage to the upper layer of mucosa can be observed in the intesti-

nal epithelium (20). These morphological changes in the small bowel epithelium are found even in the absence of cirrhosis (21). Alcohol increases intestinal permeability to macromolecules whatever the degree of hepatic dysfunction (22). The primary effect of alcohol may be the release of pro-inflammatory mediators such as tumour necrosis factor-α and IL-6 that can increase intestinal permeability and local immunoglobulin secretion. *Seillès* et al. observed too that serum secretory IgA and free secretory component concentrations were significantly increased in patients with chronic alcoholic liver disease, even at a very early stage of the disease, and decreased after alcohol withdrawal (23).

Alcoholic liver cirrhosis patients

In alcoholic liver cirrhosis patients, the observation of an increase in both serum IgA1 and IgA2 concentrations with frequencies of 57% and 67% respectively, as well as the positive correlation with IL-6 concentration, seems to be a major abnormality due to an immune response against many intestinal antigens and cytokine production. IL-6 is responsible for subsequent activation of B cells, resulting in their differentiation into IgA-secreting plasma cells.

The marked increase in total serum IgA concentrations in patients with cirrhosis could be due to a secondary effect of the initial release of free secretory component/secretory IgA to the plasma compartment, leading to abnormalities in cytokine regulation (24–26). In addition, the abnormal permeability of the intestinal barrier in alcoholic liver cirrhosis patients could allow an increased antigenic load in the plasma as food antigens and lipopolysaccharide (27). Increased IgA synthesis may reflect this increased antigenic load and the diminished T-cell suppression, or T-cell independent B-cell stimulation (8, 28). The IgA2 antibodies also have lipopolysaccharide and other amphiphilic components of *Gram*-negative bacteria as targets (29–30). Moreover, peripheral blood mononuclear cells from patients with alcoholic liver cirrhosis show an increased lipopolysaccharide-induced IL-6 secretion, which has been correlated with increased IgA serum concentrations (10, 26).

Recently, *Guillemin* et al. have shown that IgA faecal output was increased in alcoholic liver cirrhosis patients in comparison with control social drinkers (31). This observation confirms the possible role of the gut-associated lymphoid tissue in the serum IgA subclass metabolism. Intestinal IgA synthesis may be stimulated in alcohol liver cirrhosis patients and could help explain the serum IgA2 subclass origin also. This IgA2 subclass formed the major subclass contributing to the continuous pattern of IgA deposition in hepatic tissues (8). The role of this subclass seems very important in the extent of the liver damage and its serum increase may be related to alcohol consumption.

Viral hepatitis patients

In viral hepatitis patients, while there is an increase in serum IgA2 subclass, the absence of any difference in the IgA2/IgA1 ratio from the control social drinkers suggests that the IgA metabolism is different in heavy alcohol drinkers and alcoholic liver cirrhosis patients. The pro-inflammatory cytokines (IL-6) induce IgA secretion in both subclasses. The lower significance of the serum IL-6 increase in viral hepatitis patients than in alcoholic liver cirrhosis patients may be explained by the marked heterogeneity of this group (nature of the virus, severity of the infection). In fact, in viral hepatitis patients, there are probably only slight disturbances in the intestinal permeability, but the IgA metabolism seems to be profoundly disturbed.

Conclusion

The measurement of serum IgA sbuclasses and IgA2/IgA1 ratio in patients with either normal or elevated serum total IgA could provide a means to detect alcoholic liver patients that are heavy alcoholic drinkers or viral hepatitis patients evolving to clinical complications such as cirrhosis or damage to the gastro-intestinal tract. In these situations, the IgA2 subclass, and the IgA2/IgA1 ratio in particular, could be specific markers of chronic alcohol abuse directly related to the extent and duration of the alcohol abuse. However, further studies are required to confirm this, including a longitudinal study in heavy drinkers evolving to cirrhosis.

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