Clin. exp. Immunol. (1991) **85**, 80–84

ADONIS 000991049100198A

Naturally occurring anti-interferon-α2a antibodies in patients with acute viral hepatitis

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(Accepted for publication 23 January 1991)

SUMMARY

The occurrence of antibodies against recombinant human interferon-α2a (IFN-α2a) in patients with acute viral hepatitis (AVH) was examined by ELISA. Naturally occurring IgG anti-IFN-α2a were found in 50% of patients with type A, 50% of those with type B and in 8·3% of those with non-A, non-B AVH. The corresponding frequencies of IgM antibodies were 80%, 30% and 33·3%, respectively. IgM anti-IFN-α2a were found more frequently in patients with AVH type A than in normal control subjects ($P < 0.01$). Anti-IFN-α2a were detectable at the highest frequency 3 weeks after acute onset and then became negative. An absorption experiment revealed that IgM anti-IFN-α2a did not cross-react with recombinant human IFN-α2b. Immunoblotting analysis confirmed the binding of antibodies to IFN-α2a. Sera positive for IgG and/or IgM anti-IFN-α2a were unable to neutralize IFN-α2a. The appearance of anti-IFN-α2a was not correlated with disease severity. There was no evidence to suggest that anti-IFN-α2a impaired the elimination of hepatitis virus. This is the first study to demonstrate the occurrence of anti-IFN-α2a in patients with AVH. Detection of anti-IFN-α2a may be useful for clarifying any underlying immune events in various diseases.

Keywords acute hepatitis anti-interferon antibodies autoantibodies interferon viral hepatitis

INTRODUCTION

Interferon (IFN) has anti-viral and immunomodulating properties. Occurrence of antibodies against IFNs has been reported during the course of acute viral infections, malignancies and autoimmune diseases (Panem, 1984; Pozzetto *et al.*, 1984; Caruso *et al.*, 1990). These antibodies have also been reported after IFN therapy and may be partly responsible for resistance to the therapy (von Wussow *et al.*, 1987; Steis *et al.*, 1988). Recently, we developed a sensitive ELISA for antibodies (anti-IFN-α2a) against interferon-α2a and reported a high frequency of naturally occurring anti-IFN-α2a in autoimmune chronic liver disease (Ikeda *et al.*, 1989).

Several lines of evidence suggest that IFN plays a role in elimination of hepatitis virus in acute viral hepatitis (AVH) (Levin & Hahn, 1982; Zachoval *et al.*, 1986). The anti-IFN antibodies may modify both disease severity and outcome. Here we examined the occurrence of anti-IFN-α2a in patients with AVH, using ELISA.

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SUBJECTS AND METHODS

Patients

Sera were obtained from 42 patients with AVH after presentation. Thirteen of the patients were positive for IgM-anti-hepatitis A virus antibody (anti-HAV) and were diagnosed as having AVH type A, 17 patients positive for hepatitis B surface antigen (HBsAg) were considered to have AVH type B, and 12 patients negative for HBsAg, anti-hepatitis B core antigen (anti-HBc) and IgM anti-HAV were considered to have acute non-A, non-B AVH including four post-transfusion cases. Serum samples were also obtained from 23 healthy volunteers negative for HBsAg and anti-HBc, in whom liver function tests gave normal results. The sera were collected and stored at −20°C until use.

ELISA

ELISA was done essentially as described previously (Ikeda *et al.*, 1989). Polystyrene microtitre plates (Nunc, Roskilde, Denmark) were coated overnight at 4°C with recombinant human IFN-α2a (20 μg/ml), kindly provided by Hoffmann-La Roche (Kamakura, Japan). After washing with 0·135 M NaCl and 0·015 M Tris-HCl (pH 7·6) containing 0·05% (v/v) Tween 20

(TBS–Tween), the remaining protein-binding sites were blocked with 1% bovine serum albumin (BSA) in 0·15 M Tris–HCl (pH 7·6) (blocking buffer). The solution was removed and then 100 μl of test serum diluted 1/250 in blocking buffer were incubated in the coated wells overnight at 4°C. The wells were then washed thoroughly with TBS-Tween. One-hundred microlitres of peroxidase-conjugated goat anti-human IgG or IgM (Cappel Laboratories, West Chester, PA) diluted 1/2000 in blocking buffer were added to each well. The plates were then incubated for 1 h at room temperature and washed again. One-hundred microlitres of an enzyme substrate solution containing 40 mg of *o*-phenylene diamine and 40 μl of 30% (v/v) H_2O_2 in 100 ml of 0·1 M citrate–0·2 M phosphate buffer (pH 4·8) were added to each well. The reaction was stopped after 5 min with 100 μl/well of 2 *N* H_2SO_4. The optical density of each well at 492 nm (OD_{492}) was determined in an automatic microplate reader (MR-600, Dynatech) using dual-beam wavelengths of 630 and 492 nm. The results were expressed at OD_{492}. In order to assess non-specific binding of immunoglobulin in test sera, uncoated wells were blocked directly with blocking buffer instead of being coated with recombinant human IFN-α2a, then processed in exactly the same way as described above and used as control wells. Each test was run in duplicate. The ELISA count was obtained by subtracting the mean OD_{492} of each control well from that of each test well. The upper and lower limits of the normal range were defined as 2 s.d. above and below the mean, respectively, which was obtained in the tests of sera from healthy control subjects. The ELISA counts above the mean ± 2 s.d. of healthy control subjects were considered positive.

Absorption of anti-IFN-α2a with IFNs

Six microlitres of the serum positive for anti-IFN-α2a were incubated overnight at 4°C with various amounts of recombinant human IFN-α2a or IFN-α2b. Subsequently, one-fifth volume of 12% polyethylene glycol 6000 was added to the mixture, which was then left overnight at 4°C and centrifuged at 10000 ***g*** for 20 min. The supernatant was subjected to ELISA for anti-IFN-α2a. The final dilution of the serum was 1/250.

Anti-viral assay

The mixture of IFN-α2a and diluted test serum was assayed for anti-viral activity according to the method of Shiozawa *et al.* (1986). Briefly, the mixture was added to preformed FL cell monolayers, and after incubation, Sindbis virus was added. After additional incubation, neutral red dye was added 2 h before harvesting. Then the amount of dye taken up by the cells was determined. Neutralizing antibody was considered to be positive if the test serum neurtalized the viral-protective effect of IFN-α2a.

Immunoblotting

Two micrograms of IFN-α2a were applied to a 17% polyacrylamide slab gel under reducing conditions and then transferred electrophoretically to nitrocellulose paper according to the method of Towbin, Staehelin & Gordon (1979). The reactivity of IFN-α2a with test serum was detected using peroxidase-conjugated anti-human IgG or IgM (Ikeda *et al.*, 1989).

Statistical analysis

Statistical evaluation was performed by Student's *t*-test and χ^2 test with Yates' correction for small numbers.

RESULTS

Immunoblotting of IFN-α2a

Nitrocellulose strips were reacted with serum from a patient positive for IgG and IgM anti-IFN-α2a (Fig. 1). The reactivities were detected using peroxidase-conjugated anti-human IgG or IgM.

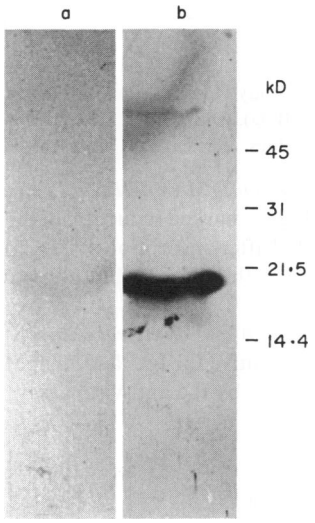

Fig. 1. Immunoblotting of recombinant human IFN-α2a. The reactivities of serum from a patient with acute viral hepatitis type A positive for IgG and IgM anti-IFN-α2a were detected using peroxidase-conjugated anit-human IgG (a) and IgM (b). The molecular masses of standard proteins are indicated. Note reaction bands in the area indicating a molecular mass of 18 kD.

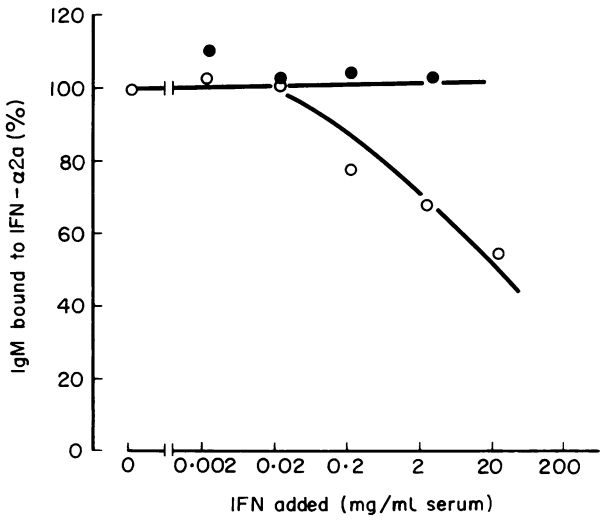

Fig. 2. Efect of absorption of serum on IgM anti-IFN-α2a levels. Serum from a patient with acute viral hepatitis type A was absorbed with recombinant human IFN-α2a (○) and -α2b (●). Marked reduction of IgM anti-IFN-α2a levels was noted after absorption with IFN-α2a but not with IFN-α2b.

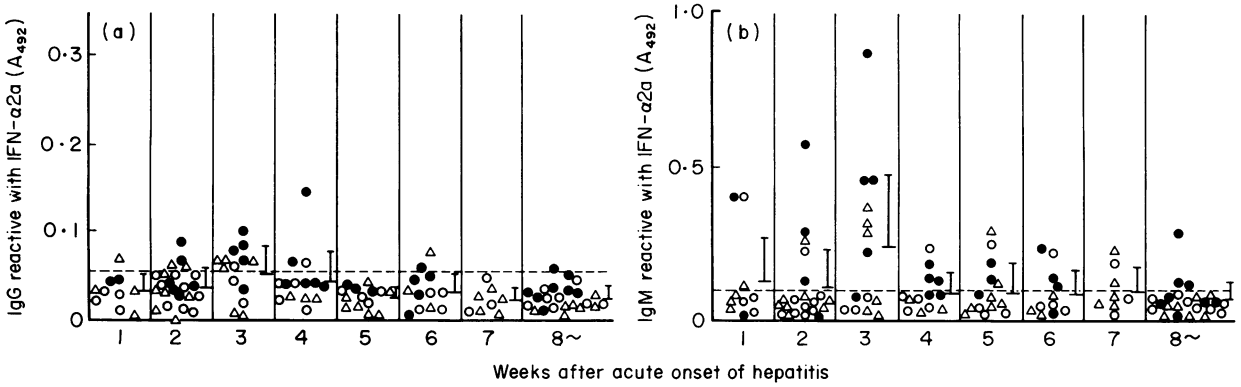

Fig. 3. Serial study of IgG (a) and IgM (b) anti-IFN-α2a antibody levels in patients with acute viral hepatitis type A (●), type B (△) and non-A, non-B (○). Anti-IFN-α2a levels were determined by ELISA. Horizontal broken lines represent the mean ± 2 s.d. for upper levels of normal values. Vertical bars represent the mean and the mean ± s.d. in each group.

Effect of absorption on IgM anti-IFN-α2a levels

As indicated in Fig. 2, marked reduction in the level of IgM anti-IFN-α2a was noted after absorption of the serum from a patient with AVH type A with IFN-α2a, but not with IFN-α2b.

Comparison of ELISA and anti-viral assay

All of the 20 sera positive for IgG and/or IgM anti-IFN-α2a by ELISA were negative by the anti-viral assay.

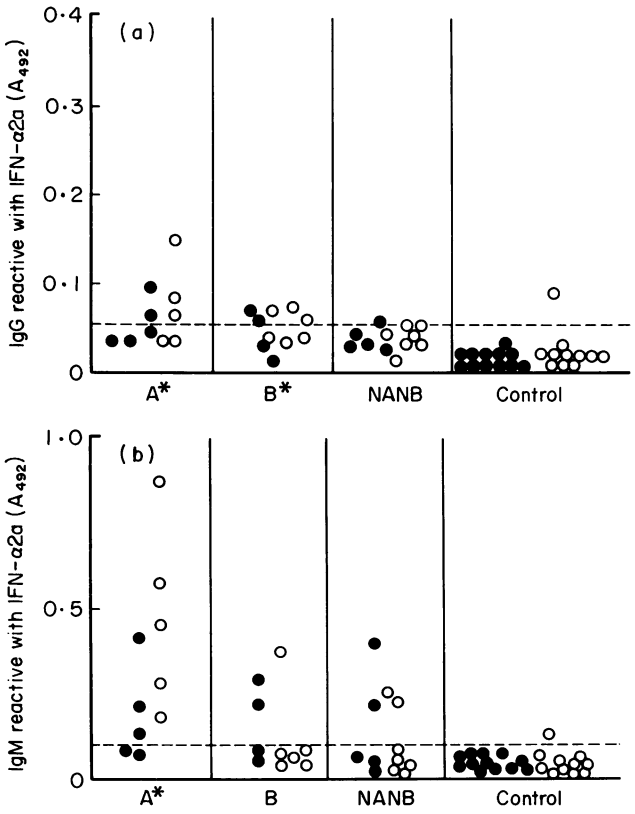

Fig. 4. IgG (a) and IgM (b) anti-IFN-α2a antibodies in normal control subject and patients (●, women; ○, men) with acute viral hepatitis types A, B, or non-A, non-B (NANB). The peak level of anti-IFN-α2a during the course of acute viral hepatitis in each patient is shown. Horizontal broken lines represent the mean ± 2 s.d. for normal values. *Significant difference in the mean level compared with that of normal subjects, Student's *t*-test ($P < 0.01$).

Studies of patients' sera

Serial studies showed that the average titres of anti-IFN-α2a were highest in week 3, and then dropped progressively (Fig. 3). Thirty-two out of 42 patients could be followed up for long enough to evaluate appearance of the antibodies. Their peak levels of anti-IFN-α2a are shown in Fig. 4. There were no significant differences in the frequencies of IgG and IgM anti-IFN-α2a among the three groups of AVH patients (Fig. 5 and Table 1). The frequencies of IgG and IgM anti-IFN-α2a were higher in patients with AVH type A than in normal subjects ($P < 0.01$). The frequency of IgG antibody was higher in AVH type B patients than in normal subjects ($P < 0.01$). Some patients

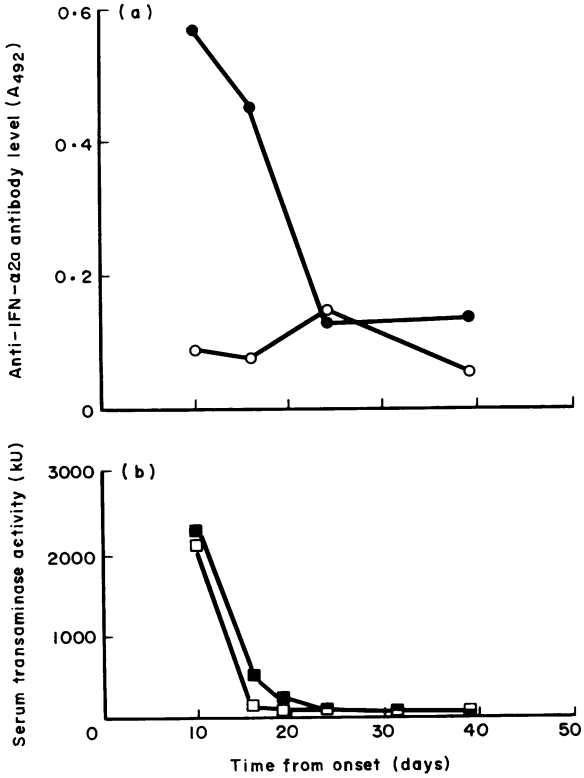

Fig. 5. Time-courses of IgG (○) and IgM (●) anti-IFN-α2a antibody levels (a) and asparate aminotransferase (□) and alanine aminotransferase activities (■) (b) in serum from a patient with acute hepatitis type A.

Table 1. Frequency of anti-IFN-α2a in sera from subjects with acute viral hepatitis (AVH) and controls

Diagnosis	No. tested	Frequency of anti-IFN-α2a (%)		
		IgG	IgM	IgG+M
AVH type A	10	50*	80*	90*†
AVH type B	10	50*	30	60*
AVH non-A, non-B	12	8·3	33·3	33·3
Healthy subjects	23	4·3	4·3	8·7

† Significant difference in frequency compared with healthy subjects ($P < 0.01$) and non-A, non-B AVH (†$P < 0.05$) by χ^2 test.

produced both IgG and IgM anti-IFN-α2a, but others produced only the IgG or IgM type alone during AVH (Fig. 5).

The peak levels of serum aminotransferase activity were recorded between 2 and 27 days after onset ($10{\cdot}75 \pm 5{\cdot}89$ days) and the peak levels of IgG and IgM anti-IFN-α2a were recorded between 5 and 46 days ($15{\cdot}5 \pm 4{\cdot}44$ and $19{\cdot}2 \pm 12{\cdot}62$ days, respectively), indicating the occurrence of anti-IFN-α2a during acute liver damage. IgG and IgM anti-IFN-α2a disappeared within 4 weeks in 88·8% and 16·7% of patients with AVH, respectively, indicating a transient IgG anti-IFN-α2a response. However, IgM antibodies in some patients persisted beyond 4 weeks. Statistical analysis revealed no association between the presence of anti-IFN-α2a and other clinical factors including the peak transaminase level, IgG level, signs and symptoms used for evaluation of disease severity, and age.

DISCUSSION

In the present study, naturally occurring anti-IFN-α2a were detected frequently in patients with AVH, especially type A and type B. However, these antibodies were non-neutralizing, and therefore might not have altered the course of the disease and might not have impaired the elimination of hepatitis virus. In fact, anti-IFN-α2a were found most frequently in patients with AVH type A, which did not progress to chronic liver disease. The absorption study revealed that anti-IFN-α2a did not cross-react with recombinant human IFN-α2b. However, we reported previously that anti-IFN-α2a detected after IFN-α2a therapy did have neutralizing action, and cross-reacted with IFN-α2b (Ikeda *et al.*, 1989). Naturally occurring anti-IFN-α2a may recognize an epitope different from that recognized by the anti-IFN-α2a induced by IFN-α2a therapy. These findings suggest that non-neutralizing cross-reactive anti-IFN-α2a antibodies are possibly a characteristic feature of AVH. Caruso *et al.* (1990) reported the presence of non-neutralizing anti-IFN-γ antibodies in patients with various types of viral infection as well as in healthy normal subjects. Naturally occurring anti-IFN-α2a detected in patients with autoimmune chronic liver disease, especially primary biliary cirrhosis, were also non-neutralizing antibodies (Ikeda *et al.*, 1989). However, naturally occurring neutralizing anti-IFN antibodies have also been reported in cancer, acute viral infection and autoimmune disease (Suit *et al.*, 1983; Trown *et al.*, 1983; Panem, 1984; Pozzetto *et al.*, 1984).

In view of the important role of IFN in the immune system, tolerance should be expected. Autoantibodies other than anti-IFN have been reported to appear during the course of AVH (Tage-Jensen *et al.*, 1980; Pedersen *et al.*, 1981; Ikeda *et al.*, 1987). There are several mechanisms by which viral infection may induce these autoantibodies. For example, the virus may initiate the production of antibodies through stimulation of helper T cells and/or the bypassing of suppressor T cells. Infectious agents including hepatitis virus can induce IFN production in the host (Levin & Hahn, 1982; Zachoval *et al.*, 1986). Recently it was reported that treatment of patients with IFN often induced anti-IFN and other autoantibodies, such as anti-nuclear antibodies, anti-smooth muscle antibodies and antibodies against thyroglobulin and thyroid microsomal antigen (Mayet *et al.*, 1989), and sometimes elicited or exacerbated autoimmune disease, such as rheumatoid arthritis and autoimmune thyroiditis (Colon *et al.*, 1990). Anti-IFN-α2a may develop during the course of AVH through a similar mechanism. The frequency of anti-IFN-α2a was higher in type A and type B than in non-A, non-B AVH, suggesting that lack of activation of the IFN system may exist in patients with non-A, non-B AVH and could be a factor in the chronicity of the disease. These hypotheses must be tested in further studies.

This study has demonstrated that non-neutralizing anti-IFN-α2a are frequently detectable in patients with AVH, but that there is no evidence of any influence of anti-IFN-α2a on the clinical course and outcome of AVH. Although the exact mechanisms underlying the appearance of anti-IFN-α2a are still unclear, these antibodies may be useful as markers of events taking place in the immune regulation system.

ACKNOWLEDGMENTS

We thank Hoffman-La Roche, Japan, for providing recombinant human interferon-α2a. This work was partly supported by a grant (63570311) from Japanese Ministry of Public Welfare and Japanese Ministry of Education.

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