SEVERE NAIL DYSTROPHY ASSOCIATED WITH RETINOID THERAPY

SIR,—Vitamin A and synthetic retinoids regulate the proliferation and differentiation of squamous epithelium. This group of drugs has lately been used to treat disorders of keratinisation, including psoriasis, Darier's disease and the ichthyoses.[1] They have also been used for severe cases of acne vulgaris, lichen planus, and certain cutaneous tumours. The synthetic retinoids have a greater therapeutic index, thus reducing the hepatotoxicity associated with high doses of vitamin A. Common side-effects reported with synthetic retinoid therapy are cheilitis, exfoliation of the feet and hands, hair loss, paronychia, and pruritus.[2] Other disturbances of ectodermal tissues have not been recognised.

In a clinical trial extending over 12 weeks (to be published) of etretinate in ten patients with severe erosive lichen planus of the oral mucosa, the frequency of the above side-effects was comparable with that in previous studies. Two female patients without cutaneous or lingual manifestations of lichen planus also acquired nail dystrophy (Beau's lines). In both cases a daily dosage of 75 mg etretinate was prescribed but this had to be reduced intermittently because of the severity of side-effects. The nail dystrophy became apparent 6 weeks after the start of treatment and consisted of a horizontal depression with splitting of the nail plate. Additional horizontal lines became evident 2 months after the trial was completed and corresponded to phases of maximum dosage.

The actions of retinoids are focused principally upon tissues of ectodermal origin, and a disturbance of nail growth might be anticipated during therapy. This side-effect may have been overlooked previously in the management of dermatological disorders because nail involvement commonly occurs as an integral part of many of these. The nails of our two patients reverted to normal after the end of etretinate treatment and have remained so for a further 6 months.

It is perhaps surprising that no dental changes have been noted in children receiving long-term retinoid therapy because the ameloblasts, being of ectodermal origin, are susceptible to changes in vitamin A levels in laboratory animals.[3-5]

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INTRACRANIAL HYPERTENSION WITH ETRETINATE

SIR,—Central nervous system toxicity associated with vitamin A is well known, but synthetic retinoids seem to be rarely responsible of such side-effects.[6] We report here a case of benign intracranial hypertension due to etretinate.

A 33-year-old woman admitted with a 4 year history of typical Darier's disease. She had keratotic papules on her trunk and neck, associated with wart-like lesions on the back of her hands and ungual changes. There were no other signs or symptoms. Histological examination of a keratotic papule confirmed the clinical diagnosis. Blood cell counts and serum creatinine, transaminase, and triglyceride levels were normal.

A daily dose of 1 mg/kg of etretinate was reduced to 0·7 mg/kg after 4 weeks because of an excellent therapeutic response. At that time she complained of slight headaches, which were controlled by floctafenine. After 2 months of treatment no more papules were observed but severe pruritus, cheilitis, dryness of the nasal mucosa, and palmoplantar desquamation led to reduction of etretinate daily dose to 0·5 mg/kg. 1 month later the patient reduced the daily dose to 0·3 mg/kg because cheilitis and palmoplantar desquamation persisted. 10 days later the drug was stopped because of rapid aggravation of occipital headache, vomiting, and giddiness, followed by two episodes of loss of consciousness. Neurological examination was normal. There was no papilloedema. An electroencephalogram and isotope encephalography were normal 15 days after drug withdrawal. Headaches disappeared 2 months later.

3 months later natural vitamin A, prescribed by another physician, induced rapid recurrence of headaches, and treatment was stopped. The Darier's disease remained uncontrolled.

This observation is characteristic of acute hypervitaminosis A due to etretinate. Headaches were the first symptom and therefore must be considered as a warning of possible drug neurotoxicity. Neurological abnormalities, rarely described in association with Darier's disease, might have been predisposing factor, but 1 year after this experience, a computerised tomographic scan did not show major abnormalities. Moreover, the rapid reappearance of headaches with natural vitamin A can be considered as secondary to the slow elimination of etretinate by the liver.[7]

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NON-A, NON-B HEPATITIS FROM INTRAVENOUS IMMUNOGLOBULIN

SIR,—A 1982 review[8] of hepatitis after infusions of plasma derivatives drew attention to the high risk of transmission of hepatitis B and, more recently, non-A, non-B hepatitis by concentrates of factor VIII (antihaemophilic globulin) and factor IX. In contrast, human normal immunoglobulin (HNIg) is not usually regarded as a vehicle for viral hepatitis infection. This is commonly attributed to loss or inactivation of virus during fractionation and to neutralising antibody in the immunoglobulin. The safety record of intramuscular HNIg in this respect is impressive and has been reinforced by the sensitive screening methods to detect HBsAg in the plasma used to prepare the fraction. However, routine screening is not available for non-A, non-B hepatitis.

In a clinical trial of an intravenous HNIg developed in this laboratory for the maintenance therapy of hypogammaglobulinaemia all twelve patients developed hepatitis compatible with a non-A, non-B viral origin. Three patients had symptoms, two being mildly icteric for a short period. The remaining patients showed only mild increases in aminotransferase levels. The data were compatible with a virus infection with a minimum incubation period of 14 to 28 days. No patients in the matched control group, receiving intramuscular HNIg from our laboratory had any clinical or biochemical evidence of hepatitis. It is not likely that the hepatitis was due to the fresh plasma that some of the patients received before the trial since aminotransferase levels at the beginning of the trial were normal. The intravenous immunoglobulin was also given, outside the trial, to patients with other conditions and their response is being carefully observed with regard to hepatitis and liver function.

From the fractionation and production aspects, these preliminary results are highly significant since the source material, a modified Cohn fraction II,[9] for manufacture of HNIg is the same whether the

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end product is for intravenous or intramuscular use. Since hepatitis or associated transaminase increases after the use of intramuscular HNIg prepared by this laboratory have never been reported, it may be that a change in downstream processing of the fraction II for intravenous use is responsible for the presence of active virus in the end product.

In view of past experience with these products, these differences in the final stages of preparation may have important implications. Thus, all preparations of immunoglobulin which have not been previously validated should be tested for viral safety and modifications in the manufacture of intramuscular or intravenous immunoglobulin should be introduced only after testing. This experience does not cast doubt on the safety of the standard intramuscular preparations which have been prepared by the established cold ethanol methods for many years.

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DOPAMINE RECEPTORS DISPLAYED IN LIVING HUMAN BRAIN WITH ^{77}Br-*p*-BROMOSPIPERONE

SIR,—Biochemical studies of post-mortem brains have revealed abnormally high numbers of dopamine receptors in patients with schizophrenia.[1] Confirmation of these and similar findings in other conditions has awaited the availability of suitable γ-ray emitting ligands. Most of the interest has so far centred on the 20 min half-life ^{11}C and some success with this radionuclide has recently been achieved.[2] However, the use of ^{11}C is restricted to those centres with an "in-house" cyclotron as well as a tomographic positron scanner (ECAT). Recently, Garnett and co-workers have used ^{18}F-6-fluoro-L-dopa and ECAT scanning to display the distribution of dopamine as distinct from dopamine receptors in living man.[3]

In 1980, Huang et al[4] prepared *p*-bromospiperone (BrSp) and showed it to be a potent displacer of ^{3}H-spiperone in rats, and that the distribution in rat brain was similar to that of the dopamine receptors. Our own studies in rats indicate a striatum-cerebellum uptake ratio of as much as 10:1; and showed that displacement of the ligand by α and β flupenthixol was stereospecific.[5]

^{77}Br ($T_{½}$ = 56 d) is prepared in the Medical Research Council cyclotron by the ^{75}As (α, 2n) ^{77}Br reaction and received as irradiated target material. ^{77}BrSp is prepared by a method similar to that described by DeJesus et al.[6] The preparation and purification by high performance liquid chromatography of ^{77}BrSp takes about 4 h, and an additional 4 h is required to check the striatum-cerebellum ratio in rats. The specific activity is 200–400 Ci (7–14 TBq)/mmol. Imaging studies were done with an IGE 400T gamma camera with a Star computer system. The optimum time for imaging was found to be about 24 h after intravenous injection of the tracer.

After the administration of 6·5 mCi of ^{77}BrSp to a fully informed normal volunteer, the reconstructed images obtained after a 1 h data acquisition period with single photon emission computed tomography (SPECT) clearly show the selective uptake of ^{77}BrSp in the striata (see figure).

Thus it seems that useful clinical studies will be possible with administered doses of the order of 10 mCi (400 MBq), though accurate quantitation may not be simple, owing to scattering of the superfluous 0·5 MeV γ-ray emitted by ^{77}Br. The 56 h half-life of ^{77}Br means that investigations can be performed in centres remote from a cyclotron, but it also means that the radiation dose received by the subject is higher than that from most routine nuclear medicine procedures, though well within the annual limit for occupationally exposed workers.

Metabolic data from tracer studies in rats and normal volunteers show that most of the administered ^{77}BrSp is initially taken up by liver and lungs and excreted largely unchanged in both urine and faeces. The effective dose equivalent in a human being is about 0·3 rem/mCi administered (0·08 mSv/MBq); this has to be considered in relation to the value of the clinical or scientific information likely to be obtained.

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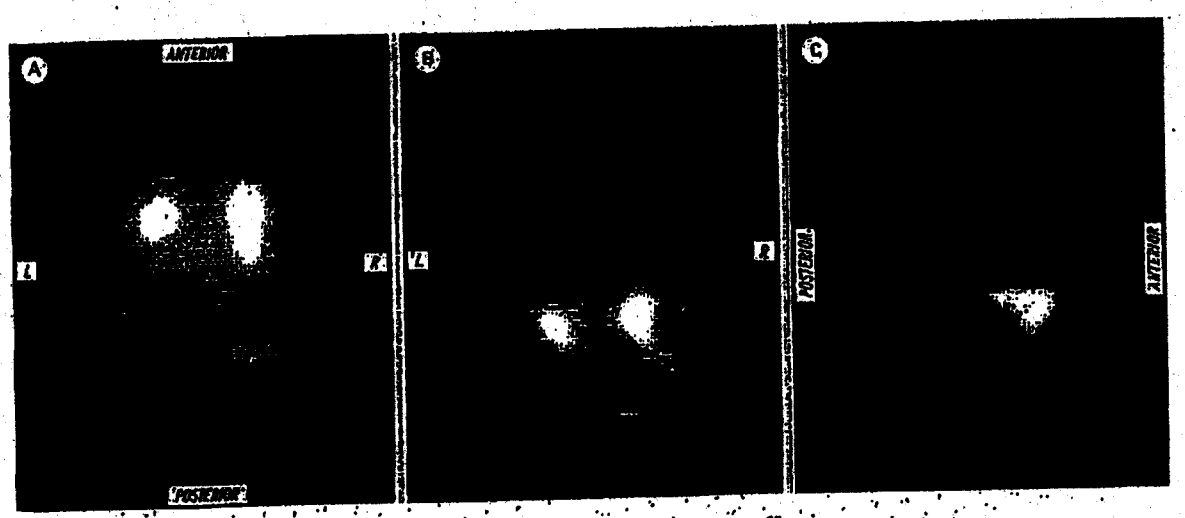

SPECT images of the head obtained 24 h after administration of 6·5 mCi ^{77}BrSp to a normal volunteer.

2 cm slices: A, transaxial; B, coronal; C, sagittal, to right of midline.

NON-A, NON-B HEPATITIS OCCURRING IN AGAMMAGLOBULINAEMIC PATIENTS AFTER INTRAVENOUS IMMUNOGLOBULIN

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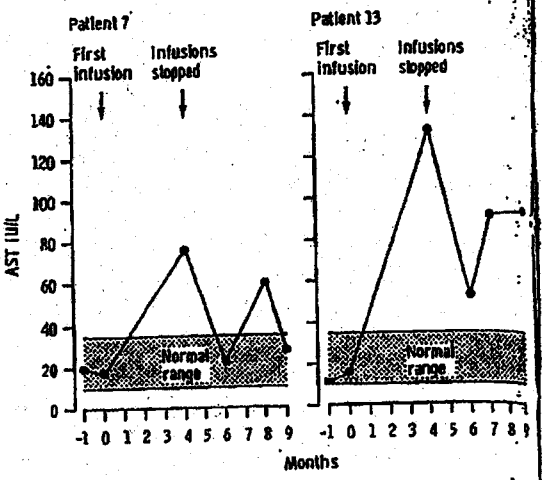
Summary Acute non-A, non-B hepatitis developed in twelve patients with primary hypogammaglobulinaemia during treatment with intravenous gammaglobulin prepared by Cohn fractionation of pooled plasma. The illness was clinically and histologically identical to the short-incubation non-A, non-B, hepatitis observed in haemophilic patients receiving factor VIII concentrates. Most of the patients were symptomless, but 10 months after onset ten of the twelve still had abnormal liver function. The occurrence of non-A, non-B hepatitis in agammaglobulinaemics indicates that humoral mechanisms are not essential for production of hepatocyte necrosis in this infection. This outbreak emphasises the need for a screening test to identify the agent in blood products, and shows that Cohn fractionation of plasma does not always inactivate the agent. Furthermore, the finding that the virus can be transmitted in IgG concentrates suggests either that the general population has a very low level of antibodies to the putative virus or that such antibodies are not virus-neutralising.

Introduction

SENSITIVE radioimmunoassays for hepatitis B surface antigen and IgM anti-HB-core allow identification of cases of post-transfusion hepatitis caused by the hepatitis B virus, and similar assays exist for the diagnosis of hepatitis A, cytomegalovirus, and Epstein-Barr virus infections which are rarer causes. Most post-transfusion hepatitis, however, is caused by a group of unidentified viruses designated non-A, non-B.[1] Serial investigations of haemophilic patients[2] and cross-challenge experiments in chimpanzees[3,4] have confirmed the existence of at least two parenterally transmitted non-A, non-B viruses with differing incubation periods. The short-incubation (2–4 weeks) type of non-A, non-B hepatitis is seen predominantly in haemophilic patients receiving factor VIII concentrates.[5] This disease is usually mild during the acute phase but a large proportion, usually greater than 80%, go on to acquire chronic lesions culminating sometimes in cirrhosis.[5] The serum transaminases fluctuate rapidly during the course of this condition and liver biopsy usually reveals a lobular hepatitis in which the mononuclear cell infiltrate is disproportionately high in relation to hepatocyte necrosis.[5] The second type of parenterally transmitted non-A, non-B hepatitis has an incubation period of 6–10 weeks. This also is a mild illness but 20–40% of patients still have abnormal liver function 6 months after onset,[6] progressing sometimes to cirrhosis. Again, the transaminases may fluctuate and liver biopsy shows chronic persistent or chronic active hepatitis with only minor lobular inflammation. This is a point of distinction from the short-incubation haemophilia-associated type of disease.

In contrast to plasma, blood, and coagulation factors, immunoglobulin preparations have rarely transmitted hepatitis. Because immunoglobulin preparations are usually prepared from large plasma pools derived from numerous donors, it has been assumed that the Cohn fractionation process either excludes or inactivates viruses.[7] We describe here an outbreak of non-A, non-B hepatitis acquired from Cohn fraction II material modified for intravenous injection. The study helps clarify the role of specific antibody in the pathogenesis of this condition.



Aspartate aminotransferase fluctuations in two representative patients.

Patients and Methods

Twenty-four patients with hypogammaglobulinaemia were recruited into an open cross-over trial to evaluate the efficacy of intravenous gammaglobulin versus conventional intramuscular immunoglobulin in preventing infection. Twelve patients were allocated to intravenous treatment, three of them with X-linked agammaglobulinaemia and 9 (2 females) with "common variable" hypogammaglobulinaemia. All twelve had previously been on regular weekly intramuscular gammaglobulin replacement therapy (25-50 mg/kg). Three had also been receiving two units of plasma every three weeks. All patients had normal serum transaminase levels at the start of the trial.

The intravenous globulin was prepared by the British Blood Products Laboratory by conventional[8] alcohol fractionation. Maltose was added to stabilise the immunoglobulin, followed by removal of the alcohol on a Sephadex G25 column and 0·2 μm filtration; the resulting solution was freeze dried. It was given fortnightly, freshly reconstituted with pyrogen-free water, at a dose of 200 mg/kg and patients were monitored for adverse effects. The trial was discontinued when hepatitis developed in some of the patients.

Peripheral-blood T cell numbers and T cell markers for helper and suppressor/cytotoxic cells were measured with commercial antibodies (Leu 1, 2, 3a) on a fluorescence activated cell sorter. Cytotoxic (NK) cell activity was assessed with a chromium-51 release assay with myeloid cells (K562) as targets, and lymphocyte/target cell ratios ranging from 1:1 to 200:1.[9] Concanavalin-A-induced suppressor function was measured in vitro with Con A concentrations of 10 μg, 5 μg, and 1 μg per well.[10] All tests were done on freshly separated peripheral-blood mononuclear cells or on samples that had been frozen in liquid nitrogen within half an hour of separation (this freezing technique has been shown not to influence either lymphocyte populations or functional assays).

Liver function tests were done before the trial and then monthly after the onset of hepatitis for a total of 10 months. In the three patients who had a liver biopsy (Menghini needle) the samples were sent for conventional histological examination and serial sections were examined for hepatitis B surface antigen. Hepatitis B surface antigen was measured in the serum of all patients with a commercial assay (Abbott).

Results

Clinical Observations

Within two weeks of the first infusion, one patient who had previously been receiving plasma experienced a "flu-like" illness and two weeks later became jaundiced with greatly raised transaminase concentrations. Hepatitis B surface antigen was absent from the blood and no virus particles were detected on electronmicroscopy of the stools. Non-A, non-B hepatitis was provisionally diagnosed. The other recipients of the intravenous immunoglobulin preparations were clinically well at this time and showed symptomatic benefit from their higher serum immunoglobulin concentrations. Therefore it was assumed that the patient with non-A, non-B hepatitis had acquired the virus from previous plasma therapy. However, 3 months after the onset of the trial, the patients were reassessed and all proved to have a raised serum aspartate aminotransferase. One patient, on close questioning, admitted to having been mildly jaundiced for about a week between infusions. Three patients then had a liver biopsy.

Six months after diagnosis of hepatitis all patients had raised transaminase concentrations and were thus, by definition, at the stage of chronic hepatitis. The transaminases have since returned to normal in two. Only two of the group ever became clinically jaundiced and most were symptomless. One patient now has unexplained marrow hypoplasia.

Laboratory Tests

The baseline aspartate aminotransferase concentrations were all normal (10–35 IU/ml), and at the first assessment after treatment all were abnormal (mean 132, range 39–545). Some patients had large fluctuations in transaminases (figure).

Hepatitis B surface antigen was never detected in any patient. Table I shows T-cell helper/suppressor ratios in the twelve patients who received intravenous gammaglobulin and in four patients who were on intramuscular gammaglobulin originating from the same plasma pool as the intravenous preparation. Where the T cell phenotype had been examined previously, this is recorded. Overall there is little or no change in subset ratios and no reversal of the ratios. Those patients who were known to have excess suppressor cells before the trial started continued to show such an excess. Concanavalin-A-induced T suppressor activity, measured in patients 2, 4, and 8, was normal (table II). NK cell activity, measured in the same three subjects, was low in patient 4 but normal in patients 2 and 8 (table III).

All three biopsy specimens showed severe lobular hepatitis with widespread mononuclear cell infiltration of the hepatic sinusoids. There was reticulin condensation indicating some liver cell loss, but in general the inflammatory infiltrate was disproportionate to the amount of liver cell necrosis.

TABLE I—T CELL PHENOTYPE

Patient	Pan-T (Leu-1) %	Helper T (Leu-3) %	Suppressor T (Leu-2) %	Ratio*	1981 ratio*
Intravenous gamma-globulin					
1	78	29	58	0·5	0·37
2	83	59	36	1·64	ND
3	29	25	19	1·3	ND
4	82	50	44	1·14	0·72
5	71	44	39	1·13	1·2
6	26	19	5	3·8	4·8
7	57	43	19	2·26	2·8
8	29	9	20	0·45	ND
Intramuscular gamma-globulin					
9	41	33	15	2·2	1·24
10	58	44	26	1·7	1·77
11	52	41	31	1·3	1·83
12	70	28	29	0·96	0·76

*Normal = 1·1–3·1.
ND = not done.

TABLE II—CON A SUPPRESSOR ACTIVITY

—	Patients 2	4	8	NR±SD
Con A concentration/well				
10 µg	0·81	1·08	1·16	1·1±0·13
5 µg	1·15	2·55	1·53	1·3±0·41
1 µg	0·63	4·39	1·64	2·9±0·7

TABLE III—NK CELL ACTIVITY (% CYTOTOXICITY)

—	Patients 2	4	8	NR±SD
Lymphocyte: target cell ratio				
200	66·3	23	61·5	69·4±13·5
100	57·5	17·5	49	74:14±7·61
50	21·1	12·3	38·9	62·5±17·6
10	12·7	4·6	15·3	28·6±9·1
1	2·5	1·0	2·9	5·1±2·4

NR = normal range.

Discussion

All the hypogammaglobulinaemic patients who received intravenous gammaglobulin acquired a short-incubation non-A, non-B hepatitis which progressed to chronic hepatitis. The rapidity of onset (2–4 weeks after infusion), the high chronicity rate, and the prominent lobular hepatitis seen on liver biopsy were reminiscent of the short-incubation non-A, non-B hepatitis seen in haemophilic patients receiving factor VIII concentrate. Probably the same virus is responsible for both conditions.

The fact that large-pool gammaglobulin preparations are capable of transmitting this type of non-A, non-B hepatitis implies that, in the community, virus-neutralising antibody occurs rarely or in extremely low titre. This has already been suggested by the observation that pooled intravenous immunoglobulin preparations do not prevent non-A, non-B hepatitis infection transmitted by factor VIII concentrates to haemophilic patients[11] and experimentally to chimpanzees.[12] Furthermore, the occurrence of severe non-A, non-B hepatitis in agammaglobulinaemic patients suggests that humoral immune mechanisms are not involved in the liver cell damage; we presume that the mechanisms are cellular or that the virus is directly cytopathic. In our patients T cell and NK function were normal and the prominent cellular infiltrate in the liver suggests their participation in the process. This notion is supported by the observation that the mononuclear cells in the livers of haemophilic patients are predominantly of the T8 phenotype (H. C. Thomas,

unpublished) which include cytotoxic T cells. There were also no significant changes in T-cell subset ratios—ie, the excess of circulating suppressor T8 lymphocytes in some hypogammaglobulinaemic patients is unlikely to be due to subclinical non-A, non-B virus infection acquired from gammaglobulin treatment.

Bone-marrow aplasia developed in one patient. This is of particular interest in that anaemia has been reported in a chimpanzee with short-incubation non-A, non-B hepatitis after injections of factor VIII concentrate.[13] Human parvovirus, which causes a failure of erythropoiesis in sickle cell anaemia,[14] can be transmitted by factor VIII concentrates[15] and can cause hepatitis. Diagnosis of parvovirus infections depends on detection of a specific IgM response which in hypogammaglobulinaemic patients is not possible. Parvovirus antigen was sought in the patient with marrow aplasia and not detected, but a parvovirus-like agent does remain a possible cause of this particular outbreak.

The apparent absence of hepatitis in patients receiving intramuscular immunoglobulin from the same pool of plasma suggests that minor variations in the manufacturing process allowed the non-A, non-B virus to remain infectious in the intravenous preparation.[16] The dose and route of administration of the infected material, and the susceptibility of the host, may also have influenced the infection rate and severity of the disease. Other intravenous gammaglobulin preparations currently available do not seem to have transmitted hepatitis, probably because viruses are inactivated by the procedures used. Various other methods have been suggested to eliminate hepatitis virus in blood products.[17,18]

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INFLUENCE OF PROPRANOLOL ON WEIGHT AND SALT AND WATER HOMOEOSTASIS IN CHRONIC LIVER DISEASE

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Summary Sixteen patients with chronic liver disease were treated with propranolol or placebo in a double-blind study. Skinfold thickness, total body water and exchangeable sodium, and urinary sodium were measured every 6 months for a year; body weight was measured every 2 (later every 3) months for 15 months. Propranolol treatment was associated with a significant rise in body weight after 9 months and significant rises in skinfold thickness and body fat after 12 months. Propranolol-treated patients showed a fall in total body water at 6 months and a rise in urinary sodium concentration at 12 months. They did not show the rise in total body exchangeable sodium that occurred in placebo-treated patients. Propranolol seems to affect salt and water homoeostasis favourably in patients with chronic liver disease.

Introduction

SODIUM retention is a characteristic of advanced liver disease, but the mechanism is unclear. Activation of the renin-angiotensin-aldosterone system, which occurs in patients with ascites,[1-4] may be important. Plasma renin levels correlate directly with the wedged hepatic venous pressure.[5] Increased sympathetic activity has been implicated in the impaired sodium and water excretion in cirrhotic patients.[6] Animal models suggest that sodium retention precedes ascites formation,[7,8] although this view is not universally supported.[9] Associated with the increase in body sodium and water is a loss of body fat and cellular mass.[10]

The effect of propranolol, advocated by Lebrec et al[11] for prevention of rebleeding from oesophageal varices in cirrhotic patients, on the renin-angiotensin system in patients with cirrhosis and ascites has been studied. Wilkinson et al showed that propranolol protected against diuretic-induced renal impairment in cirrhotic patients by blocking the rise in plasma renin activity.[12] Despite suppression of the plasma renin activity in cirrhotic patients with ascites treated with β-adrenergic antagonists, the renal excretion of aldosterone is variable, suggesting the involvement of other factors.[2] Renal sodium excretion is inversely related to aldosterone excretion before and after β-adrenergic blockade.[2]

In a study of four patients with cirrhosis and ascites, Panitch and co-workers observed a rise in urinary sodium in a patient whose renin and aldosterone levels returned to normal on propranolol.[3] Shohat et al found that propranolol increased urinary sodium excretion after an acute saline load in five cirrhotic patients with ascites.[13] This finding is

A. M. L. LEVER AND OTHERS: REFERENCES—*continued*

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MORTALITY FROM CANCER AND LEUKAEMIA IN PERSONS UNDER THE AGE OF 25 YEARS IN MILLOM AND DEPWADE RURAL DISTRICTS DURING 1959–78

Cause and time period	Millom RD			Depwade RD		
	O	E	SMR	O	E	SMR
All cancer:						
1959–67	3	4·5	67	..	..	..
1968–78	10	4·0	253*	11	5·3	209
Leukaemia:						
1959–67	1	1·6	63	..	..	..
1968–78	6	1·4	435†	8	1·8	440

O = observed number of deaths; E = expected number of deaths at age, sex and cause specific rates in England and Wales during appropriate period. SMR = 100 × (O/E) = standardised mortality ratio. ICD 7 (1959–67) and ICD 8 (1968–78) code numbers are respectively 140–207 and 140–209 for cancer, and 204 and 204–207 for leukaemia.
Significantly different from 100 at: *p<0·05, †p<0·01.

although the standardised mortality ratio in Urquhart and Cutler's table II is slightly different. The figure of 10 deaths which they give from cancer in Depwade RD during 1968–78 in under 25-year-olds is 1 short of the 11 which were coded as such. The correct figures produced on a nationally comparable basis are shown in the accompanying table. Data for 1959–67 for Depwade RD are not available, although it is known that only 2 deaths from leukaemia occurred in the age-group during those years.

I strongly endorse their final comment, that in any investigation all cases are recorded for analysis, and this we are aiming for by using every source of information available in our current case-control study in West Cumbria.

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KETOCONAZOLE IN ADVANCED PROSTATIC CANCER: DUAL PUBLICATION?

***We have received the following comment from a reader:* "The preliminary communication by Dr Trachtenberg and Dr Pont (Aug 25, p 433) is virtually the same paper rewritten as Ketoconazole Therapy in Advanced Prostatic Cancer by Dr Trachtenberg in the *Journal of Urology* (1984; 132: 61–63). Both papers give the results of treating 13 patients presenting with stage D2 prostatic cancer with high-dose ketoconazole. The paper in *The Lancet* starts off with 15 patients, but after 2 dropouts it appears to deal with the same patients. The data revealed in each paper are slightly different—for example, the time intervals for which information is given".

We asked Dr Trachtenberg and Dr Pont to respond to this point and their reply follows.—ED.L.

SIR,—We feel that the *Lancet* paper is different from and adds to the communication in the *Journal of Urology*. Therefore, both articles merit publication. Our reasons include:

(a) The 15 patients in the *Lancet* paper include the first 10 from the Toronto group and 5 never previously published cases from San Francisco. The 13 patients in the *J Urol* article are all Toronto patients.

(b) The *J Urol* paper gives results of three months' treatment; the *Lancet* paper gives six months' data.

(c) There are added biochemical, endocrinological, and radiological data in the *Lancet* article, including cholesterol values, luteinising hormone levels, prostatic acid phosphatase measured in different patients by radioimmunoassay and enzymatic assay, and a fuller delineation of effects on bone scans. In addition, the *Lancet* paper provides a comprehensive description of clinical and biochemical side effects of high-dose ketoconazole therapy.

In retrospect, we recognise that the articles appear similar upon rapid review. We regret any questions this may have raised about the primacy of data published by *The Lancet*. However, for the reasons stated, we feel that the *Lancet* report represents documentation of continuing studies rather than duplication. Given the relatively short life expectancy of patients with stage-D2 prostatic carcinoma, and the potential importance of the research, we shall continue to attempt to publish combined data from our studies at intervals of six months.

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**The need to seek this explanation from Dr Pont and Dr Trachtenberg might have been avoided if Dr Trachtenberg had informed *The Lancet* of his intention to place his separate paper in the *Journal of Urology*.—ED.L.

NON-A, NON-B HEPATITIS AND INTRAVENOUS IMMUNOGLOBULIN

SIR,—Immunoglobulin is one of the safest biological products available.[1] Since the development of the cold alcohol precipitation technique, immunoglobulin from pooled plasma has been given to millions of individuals and has become the cornerstone for management of patients with humoral immunodeficiency.[2] Modified immunoglobulin (IVIG) preparations now permit intravenous infusion of large amounts of IgG.[3,4]

Last year non-A, non-B (NANB) hepatitis was reported in 12 immunodeficient patients given IVIG[5,6] prepared by the British Blood Products Laboratory from conventional Cohn fraction II suspended in a maltose-containing medium; 12–28 days after the first infusion serum transminase levels rose, compatible with NANB hepatitis. We have had a similar outbreak in immunodeficient patients after the introduction of a new IVIG preparation.

16 patients (11 with common variable immunodeficiency, 4 with X-linked agammaglobulinaemia, and 1 with immunodeficiency and hyper-IgM; 3 females, 13 males) were enrolled during 1982 and 1983 in a longitudinal safety and efficacy study. The starting dose of 100 mg/kg per infusion was rapidly increased to 400 mg/kg every 4 weeks. In July 1984, 2 years after the study began, 1 patient had oedema, ascites, and jaundice, and, although he had had normal levels of alanine aminotransferase/aspartate aminotransferase (ALT/AST or SGPT/SGOT) at the start of the study, transaminase activities were high (ALT 100–230, AST 120–200 U/l). Tests on stored sera showed that all but 1 patient had had normal ALT and AST levels before the study. All patients were free of hepatitis A and hepatitis B antigen before and 5 months after the first infusion and IgM antibody to hepatitis A or B virus could never be demonstrated. In 6 of 7 affected patients ALT and AST levels rose 1–3 months after the first infusion of the new preparation; 1 had had elevated tranaminase levels before the study. The index case has since died of coronary artery disease and had a grossly cirrhotic liver. None of the other 6 patients has had any symptoms of hepatic disease to date but moderately increased and fluctuating transaminase levels persist. The 9 study patients who did not have raised AST/ALT levels have been followed up for two years without AST/ALT increases or evidence of hepatitis.

The increase in transaminase activity in the affected individuals was closely related in time to the first administration of one of two lots of IVIG prepared from a single lot of Cohn fraction II paste. The differences between the affected and non-affected groups were that none of the 7 patients with increased AST/ALT had received immunoglobulin intravenously during the year before the study began and that 6 of the other 9 patients had been on high dose (400 mg/kg monthly) IVIG from a different source before the study. Perhaps some of the patients who remained free of AST/ALT increases were protected passively by the IVIG given earlier. The AST/ALT levels of these 9 patients remained normal during

continued use of several other lots of this experimental IVIG preparation.

Two outbreaks of NANB hepatitis in patients with immunodeficiency receiving modified immunoglobulin intravenously raises a number of questions. Does immunoglobulin contaminated with low-dose NANB virus cause hepatitis more readily if given intravenously than intramuscularly? Could the modification procedures used to stabilise IgG increase the infectivity of the virus? The preparation causing NANB hepatitis in Britain was stabilised by the addition of maltose and the material used in our patients contained glucose. Could NANB virus-antibody complexes formed in pooled plasma (inactivating contaminating virus) dissociate during the modification and stabilisation processes? Clearly, new methods must be explored to improve safety of immunoglobulin preparations, both modified and non-modified. The detection of reverse transcriptase activity in serum and plasma contaminated with NANB hepatitis agent(s)[7] and the isolation of a virus from sera containing NANB hepatitis agent(s) using chimpanzee liver cell cultures[8] may allow the screening of each individual plasma unit and the final product. Alternatively, it may be necessary to inactivate contaminating viral agents by methods such as pasteurisation,[9] cold sterilisation,[10] or the use of low concentrations of pepsin.[11,12]

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SIR,—There has been a tendency of late to refer to all plasma fractionation methods which involve the use of alcohol as "Cohn fractionation". Dr Lever and colleagues (Nov 10, p 1062) describe the transmission of non-A, non-B hepatitis by an intravenous immunoglobulin preparation and incorrectly refer to the primary method of isolation as "Cohn fractionation", but with a reference to the method of Kistler and Nitschmann.[1] The method of IgG isolation is critically important to reports of transmission of disease with this plasma fraction.

Most manufacturers use one of two methods to isolate from plasma the IgG-rich fraction used to prepare purified immunoglobulin suitable for intravenous use—namely, those of Cohn and Oncley[2,3] (methods 6 and 9) or Kistler and Nitschmann.[1] Both involve the purification of IgG by fractional precipitation or extraction with alcohol. Cohn's method was developed in the 1940s at Harvard Medical School under the auspices of the US Government and involves the purification of IgG exclusively by fractional precipitation. In the late 1950s Kistler and Nitschmann, using a modification of Cohn and others, combined fractional precipitation and extraction. This modification, which reduces the working times, volumes, and alcohol consumption necessary to isolate purified plasma fractions while increasing yields, was developed at the central laboratory of the Swiss Red Cross Blood Transfusion Service. As noted[1] "careful attention to ionic strength is of great importance during the fractionation of precipitate A (the IgG containing fraction). Slight deviations in the ionic strength will influence yield and purity of the gamma-globulin remarkably". The Cohn process uses re-precipitation and washing steps to control ionic strength precisely. As a result, it consistently yields IgG of higher quality and purity, but yields are lower than those of the streamlined but less controlled Kistler process.

Cohn methods 6 and 9 (with modifications[4,5]) are used almost everywhere in the United States while the Kistler and Nitschmann method or variations of it is preferred by European manufacturers. In the United States, this preference is in part due to the regulatory constraints of the Food and Drug Administration, stemming from a bad experience with Cohn method 12. Method 12 involves fractional precipitation and extraction with, primarily, zinc ions,[6] and the IgG was found to transmit hepatitis.[7] Immunoglobulins produced by methods 6 and 9 have a proven record of safety with respect to the transmission of viral diseases, especially hepatitis.[8] The development of new methods for isolating IgG has been severely limited in the United States by the difficulties with lack of success in proving equivalent safety. Dr J. S. Finlayson, of the US Food and Drug Administration, has stated[9] that fraction II (the IgG-containing fraction) isolated by Cohn methods 6 and 9 from starting plasma of proven infectivity does not transmit hepatitis.

The use of alcohol in the fractionation of immunoglobulins does not itself guarantee that the preparation will not transmit disease. If departures from proven methods are used the researcher must prove that his method produces a safe preparation.

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HEPATITIS B IMMUNE GLOBULIN TO PREVENT NON-A, NON-B POST-TRANSFUSION HEPATITIS

SIR,—While Lane and Lever et al[1,2] have described cases of non-A, non-B (NANB) hepatitis after intravenous immunoglobulin, Simon[3] found that hepatitis B immune globulin (HBIG) could prevent NANB hepatitis in haemodialysis patients. Like Simon we have done a study of HBIG in the prevention of NANB hepatitis, this time in the context of heart surgery.

Patients aged 15–65 scheduled for open heart surgery and with no history of hepatitis, alcohol or drug abuse, or hypersensitivity to blood products were randomly assigned to a control or HBIG group. Patients in the treated group received 10 ml HBIG intravenously (anti-HBs titre 250 IU/ml; 'Hepatect', Biotest Pharma, Frankfurt), 5 ml being given immediately after admission to hospital and 5 ml on the day after surgery. Further infusions were

7)

Intravenous Immunoglobulin Prophylaxis Causing Liver Damage in 16 of 77 Patients with Hypogammaglobulinemia or IgG Subclass Deficiency

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Sixteen of 77 patients (21 percent) with common variable immunodeficiency or IgG subclass deficiency contracted non-A, non-B hepatitis in association with intravenous infusions of immunoglobulin. The hepatitis seemed to run a more severe course in these patients than in non-immunodeficient patients. Twelve patients had clinical symptoms, and five died with hepatitis being the cause of death in two and a contributing factor in three. Liver biopsy specimens showed early chronic active hepatitis and cirrhosis. In addition to increases in liver enzymes, 13 patients had increases in alkaline phosphatase levels. All but two patients who contracted hepatitis had been given 50 mg/kg per week or more of intravenous immunoglobulin. Lymphocyte counts, T/B cell ratios, and T-lymphocyte function did not differ between those in whom hepatitis developed and those in whom it did not develop. The hepatitis was associated with more than one batch of a Swedish intravenous immunoglobulin, the immunoglobulin being derived from United States sources as well as from European plasma. Three previous brief reports in the literature have also associated non-A, non-B hepatitis with the intravenous infusion of various immunoglobulins. Biologic materials given to patients, including immunoglobulin, should, whenever possible, be prepared so as to ensure absence of viruses.

Immunoglobulin prophylaxis is vital for patients with hypogammaglobulinemia. After years of use, intramuscular immunoglobulin has been shown to be safe, with no reports of transmission of hepatitis virus. Recently, however, a few reports have suggested the transfer of non-A, non-B hepatitis with some of the new-generation immunoglobulin preparations made for intravenous use [1–7]. At present, the mechanism whereby these intravenous preparations can be infectious is unknown, since the same source materials are used for the intravenous and intramuscular preparations. To illustrate this problem further, we describe our experience with several patients with hypogammaglobulinemia or IgG subclass deficiency in whom liver damage developed after intravenous infusions of immunoglobulin prophylaxis were administered.

PATIENTS AND METHODS

Patients. The patients with common variable immunodeficiency (CVID) or IgG subclass deficiency included in this study and the doses and type of immunoglobulin they received appear in Table I. Nineteen patients with CVID participated in a two-dose study with 25 or 100 mg/kg per week of intravenous immunoglobulin, and 17 other patients with IgG subclass deficiencies while receiving intravenous immunoglobulin were followed in separate clinical trials. Several of these patients have been described earlier [8–10]. The other 120 patients were receiving regular immunoglob-

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TABLE I **Hepatitis in 16 of 156 Patients with Immunodeficiency after Intramuscular (n = 79) and Intravenous (n = 77) Immunoglobulin Prophylaxis**

Diagnosis	Number of Patients	Location	Dose (mg/kg/week)	Mode of Injection	Source of Plasma	Hepatitis Developed
CVID	76					
	13	NYC	25–150	IV	American	3
	10	Gbg	25–100	IV	American	6
	24	Gbg	25–50	IV	Nordic	4
	1	Gbg	100	IV	Nordic	0
	1	Gbg	1000	IV	Nordic	0
	27	Gbg	25	IM	Nordic-Irish	0
IgG subclass deficiency	80					
	7	NYC	50	IV	American	1
	21	Gbg	50	IV	Nordic	2
	52	Gbg	25	IM	Nordic-Irish	0

NYC = New York City; Gbg = Göteborg, Sweden; IV = intravenous; IM = intramuscular; Nordic = from Sweden and Finland.

ulin prophylaxis, but one patient with polymyositis received 1,000 mg/kg during one week (Table I).

Immunoglobulin Preparations. In this study, we used a Swedish albumin-stabilized immunoglobulin preparation (Gammonativ, KabiVitrum, Stockholm, Sweden) given intravenously or an immunoglobulin preparation given intramuscularly (Gammaglobulin 16.5 percent, KabiVitrum). The immunoglobulin preparations are prepared using the Cohn cold ethanol fractionation procedure with 25 percent ethylalcohol. The fraction II paste for intravenous use is not freeze-dried, but dissolved and treated with DEAE-Sephadex, stabilized with glycine, albumin, and glucose, and lyophilized. The intramuscular preparation, after freeze drying of fraction II paste, is dissolved, stored, and bottled.

Immunologic Tests. Total lymphocyte counts, percentages of T and B lymphocytes, responsiveness to phytohemagglutinin and concanavalin A mitogens, and delayed-type hypersensitivity reactions to purified protein derivative of tuberculosis, Candida, Varidase, mumps virus, and Trichophyton antigens were measured as described previously [11].

The sera of the immunodeficient patients were analyzed on two to three occasions for hepatitis B surface antigen and for antibodies against human immunodeficiency virus, Epstein-Barr virus, and cytomegalovirus.

Liver Tests. The activity of asparate aminotransferase, alanine aminotransferase, and alkaline phosphatase was determined in Göteborg as recommended by the Scandinavian Committee on Enzymes [12] and in New York as described [13,14]. Hepatitis was diagnosed if aminotransferase levels were 2.5 times above the normal level (1.75 μ/liter or 62.5 U/ml, respectively) in at least two consecutive samples taken at a six-week interval or longer, without any other reason for these elevations [15]. Liver biopsy specimens were obtained in eight patients using a Menghini needle.

RESULTS

Clinical Data. Sixteen of the 77 (21 percent) patients had levels of serum aminotransferases 2.5 or more times the normal level after the initiation of intravenous immunoglobulin prophylaxis (Table I). All patients had normal levels before the study. Fourteen of these patients had been given 50 mg/kg per week or more of intravenous immunoglobulin of which five patients had 100 and one 150 mg/kg per week, and two others had received 25 mg/kg per week, also intravenously. Hepatitis developed in recipients of intravenous immunoglobulin made of plasma obtained from both United States and European sources (Table I). Increased serum alkaline phosphatase levels were present in 13 of the patients, with extremely high levels in two (Table II). Hyperbilirubinemia developed in four patients. In contrast, 27 other patients with CVID receiving 25 mg/kg per week of intramuscular immunoglobulin for one-half to three years, with a mean of 1.5 years, did not show any increased aminotransferase levels.

In two IgG subclass-deficient patients (Patients 14 and 15), increased enzyme levels developed after only two infusions at 50 mg/kg per week three weeks apart. One of these patients (Patient 15) had a sharp and brief rise initially, followed by four months with normal levels of aminotransferases and then had another sharp increase of three enzymes. The enzyme levels of 12 patients have not returned to normal; five of these 12 have died.

Four patients with increased transaminase levels had no symptoms of liver disease. Eleven had symptoms within zero to 41 months, (mean: 11 months) after the enzyme elevation was first noted. Five patients (Patients 1 to 4 and 6 in Table II) had signs of severe liver disease, e.g., ascites and edema.

Of the five patients who died, one (Patient 1) had slowly progressive deterioration of liver function for 23 months but died of an erysipelas infection. The second (Patient 2) had increased levels of transaminases for 46 months. He had both hips replaced because of aseptic osteonecrosis, but poor clinical condition due to liver disease was present only during the last five months of his life. He died of hepatic failure. The third patient (Patient 4) had a more

TABLE II Clinical Data in 16 Patients with Hepatitis

Patient Number	Sex	Age (years)	Duration of Immunodeficiency (years)	Duration of Ig Prophylaxis (years) IM	IV	Peak Values for Abnormal Liver Enzyme Levels* ASAT	ALAT	ALP	BIL	Duration of Raised Aminotransferase Levels (months)	Subclinical Period (months)	Presence of Clinical Symptoms	Histopathologic Findings†
CVID													
1	F	73	16	11	3	3.1	1.3	9.5	65	23	3	+ dead	
2	M	43	30	25	4	13	7.4	9.9	504	46	41	+ dead	
3	M	57	29	24	4	20	14	47	80	7	Md	+ dead	CAH, CIRR
4	M	45	12	2	2	6.0	5.0	97	—	18	9	+ dead	1.CAH 2.CIRR (autopsy)
5	M	52	22	8	3	12	9.9	110	33	28	9	+ dead	1.AH 2.CIRR
6	M	20	3	1	2	3.6	4.6	21	—	30	0	+	1.AH 2.AH 3.AH CAH?
7	M	21	12	8	3	4.6	10	6.2	—	20	20	−	CAH
8	F	42	22	18	4	2.4	2.8	—	—	28	28	−	AH
9	M	23	13	7	6	199	50	94	—	13	13	−	
10	M	52	15	7	6	960	607	437	—	14	0	+	
11	M	36	10	5	3	250	469	140	—	3	0	+	
12	M	47	15	5	2	1.9	4.5	5.4	—	8	Md	+	
13	F	37	11	6	5	11	7.7	13	—	2	2	−	
IgG subclass deficiency													
14	F	33	2	1	¼	1.4	3.4	—	—	10	0	+	CAH
15	M	33	2	—	¼	9.8	14	5.5	—	7	3	+	AH
16	F	10	2	0	1	93	81	—	—	3	4	+	

IM = intramuscular; IV = intravenous; ASAT = asparate aminotransferase; ALAT = alanine aminotransferase; ALP = alkaline phosphatase; BIL = bilirubin; Md = missing data (liver enzyme levels were not obtained before clinical symptoms appeared); CAH = chronic active hepatitis; CIRR = cirrhosis; AH = acute hepatitis.

* Upper limits of normal sera: in Göteborg, ASAT and ALAT equaled 0.7 μkat/liter, ALP equaled 5 μkat/liter, and BIL equaled 21 μmol/liter. In New York City (**bold face**), ASAT and ALAT equaled 25 U/ml, ALP equaled 88 U/ml, and BIL equaled 1.0 mg/dl.

† Consecutive biopsies indicated by numbers.

rapid progression of liver disease with edema and ascites. He died suddenly and unexpectedly at home. Autopsy suggested vascular collapse as the cause of death. The fourth patient who died (Patient 3) showed a very severe and rapid course with profound fatigue, hypotension, diarrhea, jaundice, ascites, and intermittent fever. During the last nine days of his life, he was treated with 5×10^6 IU units of alpha interferon per day (Introne-A, Schering Corp., Kenilworth, New Jersey). Results of his liver function tests improved dramatically, but he died of bilateral pneumonia. One additional patient (Patient 5) received alpha interferon for four months. He showed clinical improvement and the liver enzyme levels decreased. When the alpha interferon treatment was stopped, his aminotransferase levels increased rapidly and the patient's condition deteriorated. A second course of alpha interferon therapy had no effect and the patient died.

Two additional patients have had increased liver enzyme levels, but not 2.5 or more times the upper normal limit. Enzyme levels for one of these patients have normalized and results of a subsequent liver biopsy were normal. One additional patient with CVID, who had polymyositis and received 1,000 mg/kg of immunoglobulin during one week, later had increased aminotransferase levels (data not shown). However, results of the liver biopsy was normal.

Histopathologic Evaluation. A liver biopsy was performed in eight patients. In four, the histology showed chronic active hepatitis with varying degrees of inflammation and aggressivity; in one of these patients, early cirrhosis developed (Patient 3, Table II). Another patient who also had micronodular cirrhosis at autopsy had a few months earlier only some fibrosis on biopsy (Patient 4). Patient 5 had initially had a mild acute hepatitis, but died six months later with cirrhosis.

Liver samples from four patients showed acute hepatitis. One originally had a clinical picture of acute viral hepatitis with necrosis of single hepatocytes and a moderate inflammatory reaction. One year later, a few necrotic hepatocytes could still be seen, but the inflammatory process had changed to more chronic active hepatitis of the early aggressive type (Patient 6). One patient had an acute hepatitis (Patient 15). Patient 8 was classified as having mild acute hepatitis with very discrete inflammatory reactions consistent with unspecific reactive processes.

The microscopic examination showed the common features of active hepatitis as seen in non-immunodeficient patients. No plasma cells could be seen and no lymph follicles were observed in the chronic cases. A pronounced Kupffer cell proliferation seemed to occur with an increased number of lymphocytes within the lobules.

Immunologic Investigations. No differences were noted between the hepatitis diseased and healthy patients in regard to total leukocyte and lymphocyte counts, B cell, T cell, CD3-, CD4-, or CD8-positive cell percentages, mitogen responsiveness to phytohemagglutinin and concanavalin A, or in delayed-type hypersensitivity reactions. All patients tested negative against hepatitis B surface antigen and no changes in antibody titers against human immunodeficiency virus, Epstein-Barr virus, or cytomegalovirus were noted.

COMMENTS

This study shows that severe and sometimes lethal hepatitis developed after prophylaxis with an immunoglobulin preparation for intravenous use in antibody-deficient patients. The increased aminotransferase concentrations and the histopathologic picture in our patients are consistent with a drug- or virus-induced hepatitis, which is supported by studies in chimpanzees [15]. However, increased serum levels of alkaline phosphatase are not usually part of the pattern of that disease [16], but other or additional forms of viral hepatitis cannot be excluded serologically in patients with hypogammaglobulinemia.

In patients with antibody deficiency, severe forms of viral hepatitis may develop when infection with hepatitis B virus occurs [17]. Chronic active disease, with or without cirrhosis, has been reported after acute hepatitis in patients with agammaglobulinemia or hypogammaglobulinemia [18], but there is also a report of an asymptomatic carrier state in a patient with X-linked hypogammaglobulinemia [19]. In three of our patients, hepatitis contributed to death. One patient died of hepatic failure and chronic active liver disease verified by microscopy developed in four patients. In four of the 16 patients, however, the liver disease was asymptomatic.

It was notable that hepatitis occurred in three patients after only two intravenous infusions of immunoglobulin of 50 mg/kg per week, three weeks apart. Two of these patients were IgG subclass deficient (Patients 14 and 15). In other patients, higher doses and a larger number of infusions had been given for a much longer time before the liver damage appeared. The mean initial period without clinical symptoms was at least 11 months. Hepatitis developed in recipients of different batches of intravenous preparations made from United States sources as well as from European plasma.

No case of hepatitis was observed among the patients given intramuscular immunoglobulin, although this preparation was made from the same plasma pools as the intravenous immunoglobulin. It is not clear whether the absence of hepatitis in patients receiving intramuscular immunoglobulin prophylaxis is due to the different final steps used in the production procedures for the intravenous as compared with the intramuscular form or to the different routes of administration [20]. It could also be a matter of the dose; intravenous immunoglobulin was given in larger doses than was intramuscular immunoglobu-

lin. It could also be that the hypogammaglobulinemia and the often impaired T-lymphocyte function in these patients [21,22] made them an easier target for a drug-carried virus. However, hepatitis developed in three patients with only IgG subclass deficiency and normal T-cell function, and no differences in immunologic parameters were noted among hepatitis diseased and healthy patients with CVID. Two patients were treated with alpha interferon with some success [23], in agreement with a recent report [24].

The intravenous immunoglobulin preparation used here has been successful in preventing infections in CVID patients, has been easy to administer, and has caused few side effects in previous studies [9]. It is tragic that this useful drug has caused hepatitis. It is urgent to determine the mechanisms involved since our experience is not unique [1–7]. To avoid similar incidents in the future, it seems necessary to include a step in the production of immunoglobulin preparations that eliminates viruses. Such procedures have been applied to a few intravenous immunoglobulin preparations, one of which we are currently testing.

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Immunoglobulin Transmits Hepatitis C. True or False?

To the Editor:

In their interesting review, Heintges and Wands[1] wrote: "... HCV-RNA was detectable in more than one-half of the intramuscular preparations of immunoglobulins. Thus, patients with immunoglobulin deficiency and who received such prophylactic antibody preparations frequently developed chronic HCV infection."

However, Heintges and Wands cited a study by Bjøro et al.,[2] who reported that patients with primary hypogammaglobulinemia who received intramuscular immunoglobulins for long periods of time never acquired hepatitis C virus (HCV) infection. Only a group of patients who received a batch of intravenous immunoglobulin contaminated with non A, non B hepatitis virus acquired the infection.

Clearly, there is a need to clarify the topic of immunoglobulin administration and HCV transmission also in view of the medical, scientific, and legal aspects.

IMMUNOGLOBULIN PREPARATIONS

Standard or "polyvalent" immunoglobulin is prepared from blood pooled from at least 1,000 donors. Immunoglobulin preparations contain a wide range of antibodies resulting from infections widely spread in the population or from vaccinations. Hyperimmune globulin is prepared from the blood of a smaller number of donors appropriately vaccinated or convalescent from a given disease; hence hyperimmune globulin contains the same range of antibodies as standard immunoglobulin, but one antibody is much more concentrated than the others (at least 5-fold). After injection, the antibodies are present in the bloodstream and in interstitial fluids where they bind specifically to the various infectious agents (antigens) to form immune complexes that then are eliminated via the reticular-endothelium cell system. Antibodies do not enter the cells, and they have a half-life of 21 to 25 days. Thus, their protective effect can last 2 to 3 months.

Since 1971, hepatitis B surface antigen–positive blood units have not been included in immunoglobulin starting material. Since 1985 and 1992, also anti-human immunodeficiency virus-1– and anti-human immunodeficiency virus-2–positive units, respectively, have been discarded. In the early 1990s, most developed countries forbade the use of anti-HCV–positive blood for immunoglobulin products (e.g., 1990 in France, 1991 in the United States, and 1993 in Italy).

INTRAMUSCULAR IMMUNOGLOBULIN

Intramuscular immunoglobulin preparations are prepared according to the Cohn fractionation process, which separates the fraction containing antibodies that neutralize various infectious agents. The resulting preparations are highly concentrated (16% in solution and containing 160 mg of protein/mL). Other manufacturing procedures do not ensure the same safety.[3]

Over the last 50 years, many millions of individuals worldwide have received intramuscular immunoglobulin without contracting infections. Intramuscular immunoglobulin prepared according to the Cohn process has been proclaimed safe by the Centers for Disease Control[4,5] and by the World Health Organization.[6]

Recently, concern was aroused when 50% of batches of unscreened intramuscular immunoglobulin, both standard[7] and hyperimmune,[7,8] tested positive for HCV-RNA. This led to the suggestion that patients with chronic hepatitis C infection could have been infected by a previous inoculation of intramuscular immunoglobulin. We were able to provide the first direct evidence that HCV infection is not transmitted by intramuscular immunoglobulin containing HCV-RNA. In fact, in a randomized controlled trial 450 at-risk sexual partners (mean age: 43.8 years) of HCV-infected individuals received 4 mL of unscreened intramuscular immunoglobulin every 2 months for a mean of 13.5 months. A total of 3,260 doses of immunoglobulin were administered, about 50% of which were HCV-RNA positive, and none of the immunoglobulin recipients monitored at 4-month intervals became HCV infected.[9,10]

Similarly, in an uncontrolled trial that started at the end of 1989,[11] we treated 78 at-risk sexual partners (mean age: 29 years) of HCV-infected subjects for about 6 years according to the same protocol.[9] The partners received unscreened intramuscular immunoglobulin (about 50% were HCV-RNA positive) until March 1993, when testing of blood units for anti-HCV became mandatory in Italy. Thenceforth, the sexual partners received screened immunoglobulin preparations. The study was stopped in July 1995, when it was first demonstrated that the "new" screened commercial intramuscular immunoglobulin lacked anti-gpE1/E2 neutralizing antibodies, whereas the "old" unscreened commercial intramuscular immunoglobulin contained high titers of these antibodies.[9,12] No sexual partner of this study became HCV-RNA positive.

The safety of HCV-RNA–positive intramuscular immunoglobulin preparations can be attributed to several factors: (1) partitioning of viruses away from immunoglobulin, (2) inactivation of viruses by the fractionation process, and (3) a high concentration of neutralizing antibodies.[7,9,12]

Since 1995, it has been recommended to perform HCV-RNA testing on the final product[13,14] or on the starting plasma pools[15] with respect to intramuscular immunoglobulins that have not undergone any HCV inactivation process following Cohn fractionation process.

INTRAVENOUS IMMUNOGLOBULIN

Intravenous immunoglobulin is 5% solution of normal or specific immunoglobulin (the concentration of the latter can be even higher) that undergoes an additional preparation process to be administered by the intravenous route.

Although intramuscular immunoglobulin has never been associated with HCV transmission, from 1983 to 1994 at least 8 outbreaks of non A, non B/HCV infections occurred, 7 outside the United States and 1 inside the United States, in subjects who received intravenous immunoglobulin. During each outbreak, the number of HCV-infected patients varied from 1 to 28.[16] In 1994 an outbreak of HCV infection was associated with intravenous immunoglobulin (Gammagard) produced by Baxter Healthcare Corporation (BHC), Deerfield, IL.[13] The first cases occurred in the United Kingdom, Spain, and Sweden. Successively, 110 cases were reported in the United States.[17] It is noteworthy that, at that time, Gammagard was produced without any of the additional HCV-

inactivation processes that later came into use.[13,18] To explain this outbreak, it was suggested that, after the introduction of blood screening for anti-HCV and consequently the exclusion of anti-HCV–positive blood units, the starting blood pool could have contained blood from donors in an early stage of disease, *i.e.*, before the patients became anti-HCV positive. It was also speculated that a hypothetical neutralizing antibody could have been removed with the anti-HCV–positive blood units.[19,20]

In this context, it is interesting to recall a recent study in which plasma containing infectious HCV incubated with experimental intravenous immunoglobulin prepared from about 200 anti-HCV–positive blood donors did not cause infection in the chimpanzee, whereas the same infectious plasma incubated with commercial intravenous immunoglobulin prepared from over 1,000 anti-HCV–negative donors caused infection in the animal.[21] These results are consistent with the presence of neutralizing antibodies in the intravenous immunoglobulin from anti-HCV–positive blood and their absence from intravenous immunoglobulin from anti-HCV–negative blood.

Since 1994, most intravenous immunoglobulin products—in addition to the Cohn method—undergo stringent procedures to inactivate HCV and other infectious agents.[18,22]

Although many millions of grams of intravenous immunoglobulin are used each year, and their use is continuously increasing, no cases of HCV infection have been reported in treated subjects after the advent of new viral-inactivation procedures.[14]

There are several possibilities to explain why pre-1994 intravenous immunoglobulin resulted in some cases of HCV infection, whereas intramuscular immunoglobulin did not. (1) Intramuscular immunoglobulin is more concentrated than intravenous immunoglobulin, so that immune complexes form more easily in the former; when these complexes enter the bloodstream they are eliminated by the reticular-endothelium cells system. (2) Intramuscular immunoglobulin is adsorbed more slowly; in fact, the highest antibody titer in the blood is reached about 48 hours after injection. (3) A higher amount of immunoglobulin is injected intravenously than intramuscularly. (4) Although both types of immunoglobulin were produced with the Cohn method, the subsequent production steps differ.

In conclusion, (1) intramuscular immunoglobulin has never transmitted HCV infection; and (2) some intravenous immunoglobulin products used before 1994 caused a few cases of HCV infection, whereas intravenous immunoglobulin prepared after 1994 is totally safe.

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