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High risk of non-A non-B hepatitis after a first exposure to volunteer or commercial clotting factor concentrates: effects of prophylactic immune serum globulin

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SUMMARY. After a first exposure to factor VIII concentrates, 9/9 British patients treated with U.S.A.-derived commercial products, and 10/12 treated with British volunteer (NHS) products, developed acute non-A, non-B (NANB) hepatitis. Hepatitis following commercial products was more severe, and of shorter incubation. High previous exposure to NHS blood products seemed to prevent NHS but not commercial factor VIII-induced hepatitis; the latter was also not attenuated by administration of U.S.A.-derived commercial immune serum globulin (ISG). After a first exposure to NHS factor IX concentrates without ISG, 4/4 patients developed short incubation NANB hepatitis; one also contracted prolonged incubation hepatitis B. One patient treated with ISG and factor IX of proven infectivity did not develop hepatitis, suggesting protection by ISG. Observed differences between concentrates might be attributable to their content of different NANB agents, but dose-related effects could provide alternative explanations. This data provides a basis for comparative assessment of new products of possible reduced infectivity in only small numbers of patients.

All plasma collected in the United Kingdom is obtained from volunteer, unpaid donors. However, the quantity of factor VIII concentrate fractionated from this plasma by the National Health Service (NHS) is insufficient to meet demand. Treatment of patients with haemophilia A is therefore dependent on imported commercial concentrates, almost all of which are derived from paid donors in the U.S.A. Of the total factor VIII used in the U.K. in 1982, commercial concentrate accounted for 63%, NHS concentrate for 32%, and NHS cyroprecipitate for 5% (U.K. Haemophilia Centre Directors annual statistics, 1982). Patients

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with haemophilia B can be treated with NHS products exclusively because demand for factor IX concentrate is less than that for factor VIII.

Although it is well established that the risk of post-infusion hepatitis is higher after commercial than volunteer blood (Hollinger *et al*, 1981), the evidence that the same holds for clotting factor concentrates prepared from large plasma pools is less substantial. Acute post-infusion hepatitis in patients treated with these products is usually of the non-A, non-B (NANB) type, and there is a disturbingly high rate of progression to chronicity (Bamber *et al*, 1981; Dienstag, 1983). The overall incidence of acute hepatitis in haemophilic populations has been reported to be only 2–6% of treated patients per year, whether volunteer or commercial products have been used (Biggs, 1974; Kim *et al*, 1980; Rickard *et al*, 1982). However, patients who have been infrequently or never previously exposed to blood products are at higher risk (Kasper & Kipnis, 1972; Sugg *et al*, 1980; Norkrans *et al*, 1981; Fletcher *et al*, 1983). Also, most studies have probably underestimated risks because a proportion of patients with acute NANB hepatitis remain asymptomatic, and will therefore not be recognized unless their biochemical status is monitored prospectively. Quantitation of the risks of currently available products is of importance because it is by comparison with these risks that the efficacy of new products of possible reduced infectivity will be assessed.

Since 1978, both because of increasing awareness of the probability of underdiagnosis of acute post-infusion hepatitis, and because we wished to obtain plasma samples which might be used as sources of antigen/antibody in assays for serological markers of NANB infection (Luo *et al*, 1983), we have prospectively monitored biochemical liver function tests in patients receiving first exposures to clotting factor concentrates and cryoprecipitate, whether or not they had previously received other blood products. The very high incidence of acute NANB hepatitis observed following concentrate therapy prompted a pilot clinical study of prophylactic immune serum globulin (ISG). This report provides a quantitation of the risks of acute hepatitis associated with different blood products obtained from volunteer and commercial sources, and describes our experience with ISG.

PATIENTS AND METHODS

Patients, study design and definitions. During the 5 year period April 1978 to March 1983, 58 patients with congenital deficiencies of coagulation factors VIII or IX received 60 first exposures to factor VIII concentrate, factor IX concentrate, or cryoprecipitate at the Royal Free Hospital Haemophilia Centre. Events following 31 of these first exposures, which included five episodes in which ISG was used in addition to concentrates, were prospectively studied by serial clinical assessment and blood sampling before and after exposure. In the remaining 29 instances, problems of patient accessibility and compliance prevented the acquisition of adequate prospective data. Evaluation of outcome in this latter group was therefore retrospective, and largely limited to analysis of clinical rather than biochemical or serological information. Unless otherwise stated, data given in this report refers only to prospectively studied patients.

Only a minority of the patients were 'virgin'—although most needed infrequent treatment, a majority had received blood, plasma or cryoprecipitate therapy before their first

exposure infusions. A best estimate of previous total lifetime exposure in terms of donor units (i.e. the number of whole blood donations needed to prepare the products to which the patients were exposed) was obtained from the patients' records (Table I). Nine patients had been treated with blood products (cryoprecipitate exclusively) in the 6 month period before their first exposure infusion. In these patients it was not possible to be certain that hepatitis which followed concentrate therapy was necessarily attributable to concentrate. Since this seemed much the most likely possibility, however, it was assumed to be so in the analysis. None of the patients who developed hepatitis received additional blood product therapy between their first exposure courses of treatment and the onset of hepatitis.

Of the 30 patients who were studied prospectively (one patient received first exposures to both cryoprecipitate and concentrate), 13 had haemophilia A, four had haemophilia B, and 10 had von Willebrand's disease. Three female carriers, two of haemophilia A and one of haemophilia B, were also studied. Five patients (six exposures), were aged less than 5 years at the time of their first exposure, and nine were aged between 5 and 20 years (Table I). Of the patients with haemophilia A or B, seven were classed as haematologically severely affected, having less than 1 u/dl circulating factor VIII or IX. Treatment was given either to stop bleeding or as prophylaxis before surgery. Duration and dosage of therapy, and choice of therapeutic product, were influenced by clinical circumstances, local availability of products, and departmental policies which operated at the time treatment was given. These policies changed over the period of the study, as it became appreciated that the risk of NANB hepatitis after concentrate therapy was very high.

Blood samples were taken, and patients clinically assessed, immediately before their first exposure infusions, at 1–2 weekly intervals for the next 3 months, and at 1–2 monthly intervals for a further 6 months. Biochemical liver function tests were carried out on all blood samples, and were normal in all patients before first exposure infusions. Sera were stored frozen and selected samples from all patients retrospectively analysed for serological evidence of acute or previous viral infection. None of the patients had received hepatitis B vaccine. Patient 1 had had an attack of hepatitis B following treatment with cryoprecipitate 11 years previously, and was HBsAg/anti-HBe positive at entry to the study. Otherwise, none of the patients had serological markers of previous or current hepatitis B infection before their first exposure course. The only other patients known to have had previous hepatitis were patients 4 and 6, who had had attacks of post-infusion non-B hepatitis after treatment with cryoprecipitate and blood, respectively, several years previously.

The occurrence of acute post-infusion hepatitis was the primary endpoint of the study. This was diagnosed when three criteria were fulfilled: (1) biochemical liver function tests were normal immediately before the first exposure infusion, and were normal on any samples obtained in the 6 month preceding period; (2) aspartate transaminase (AST) levels rose to exceed $2\frac{1}{2}$ times the upper limit of normal in at least two post-infusion samples taken within 4 weeks of each other; and (3) no cause for the hepatitis other than blood product administration could be identified. *Symptomatic acute hepatitis* was defined as the occurrence, in a patient with acute hepatitis who had been previously well, of anorexia, vomiting, fever, pale stools, dark urine, jaundice, abdominal pain or pruritus. In prospectively studied patients, the presence or absence of these symptoms was established by direct questioning

and examination at the time the patient attended for a blood sample to be taken. In retrospectively studied patients the patient presented because of such symptoms. *Acute NANB hepatitis* was diagnosed by serological exclusion of acute infection with hepatitis A virus (HAV), hepatitis B virus (HBV), cytomegalovirus (CMV), and Epstein Barr virus (EBV). *Incubation period* was taken as the time interval between the first infusion of the product and the first abnormal AST result.

Therapeutic products (Table 1). All *cryoprecipitate* and *factor IX concentrate* was prepared by the NHS from volunteer donor plasma. *Factor VIII concentrates*, all described as being of intermediate purity by their manufacturers, were either made by the NHS at the Blood Products Laboratory, Elstree, England, or bought from three manufacturers, the source of plasma in the latter case being exclusively of U.S.A. origin. Donor pool sizes for concentrate were in the range 1500–5000. *Immune serum globulin* (ISG) for intravenous use was provided by Immuno Ltd, Vienna, Austria, and prepared from U.S.A.-derived plasma pools obtained from approximately 3000 donors. ISG was also used in a product prepared for this study (Kryobulin-G, Immuno, batch 1/81) which contained a mixture of factor VIII concentrate and ISG (2·55 mg ISG/unit of factor VIII). This product was used to treat three patients. Apart from the patients treated with Kryobulin-G, and two patients exposed to the same batch of NHS factor VIII concentrate, a specific batch of concentrate was only used to treat a single patient. All but five of the 26 first exposure courses of concentrate were limited to single batches of product. In these five instances, two batches of the same type of product were used in each course.

Therapeutic regimes in ISG-treated patients. Patient 6 received 45 325 u (781 u/kg) of commercial factor VIII concentrate over a 16 d period. A total of 6000 mg ISG (103 mg/kg, batch 240379) was given i.v. in divided doses of 1000 mg, starting immediately before the first infusion of factor VIII and then on days 3, 6, 9, 15 and 43 after this infusion. *Patient 7* received Kryobulin-G (11 454 u factor VIII, 164 u/kg; 29 210 mg ISG, 417 u/kg) over a 5 d period. *Patient 8* received a single infusion of Kryobulin-G (1494 u factor VIII, 26 u/kg; 3810 mg ISG, 67 mg/kg). *Patient* 9 received a single infusion of Kryobulin-G plus additional i.v. ISG batch 241480 (total doses: 1494 u factor VIII, 23 u/kg; 8810 mg ISG, 137 mg/kg). *Patient 30* received 6200 u (89 u/kg) factor IX over a 4 d period. A total of 9500 mg ISG batch 240979 (136 mg/kg) was given i.v. in four divided doses, starting immediately before the first infusion of factor IX and then on days 2, 3 and 4.

Laboratory methods. Serum IgM antibodies to HAV (IgM anti-HAV) were measured by capture assay (Abbott). Hepatitis B surface antigen (HBsAg) and antibodies to HBsAg (anti-HBs) were measured by monoclonal antibody-based solid phase radioimmunoassay (RIA) (Goodall *et al*, 1982) and by Ausab (Abbott), respectively. Serum IgM antibodies to CMV (IgM anti-CMV) were measured by RIA (Kangro, 1980). Serum IgM and IgG antibodies to the EBV capsid antigen (anti-EBV) were measured by indirect immunofluorescence (Henle *et al*, 1974).

Ethical and legal considerations. ISG and Kryobulin-G are unlicensed products in the U.K. and were used on a 'named patient' basis under the provisions of the Medicines Act, 1968. The nature of the study, and the reasons for wishing to use these products, were explained in detail to all recipients and their verbal consent obtained. The study had institutional Ethical Committee approval.

RESULTS

Incidence and features of post-infusion hepatitis

Outcome in prospectively-studied patients is shown in Table I and Fig 1. None of the five patients treated with cryoprecipitate developed hepatitis, and no patient with hepatitis had serological evidence of acute infection with HAV, CMV or EBV. All nine patients treated with commercial factor VIII concentrate, and 10 of the 12 patients treated with NHS factor VIII concentrate, developed acute NANB hepatitis. All four patients treated with NHS factor IX concentrate without ISG contracted acute NANB hepatitis and one of these patients (patient 29) also subsequently developed acute hepatitis B, the only patient in the study to do so. Symptomatic acute NANB hepatitis was more common in patients treated with commercial factor VIII (66%) than in those treated with NHS factor VIII (30%) or factor IX (25%), and it was noticeable that in symptomatic patients, clinical features of hepatitis, particularly jaundice, were more pronounced in the commercial factor VIII-treated group. However, the apparent lower incidence of symptomatic hepatitis amongst NHS factor VIII-treated patients may have been influenced by the difficulty of detection of mild symptoms in infants. Of the 29 retrospectively-studied first exposures, which included 11 to commercial factor VIII concentrate and eight to NHS factor VIII concentrate, only three patients, all of whom had received commercial factor VIII, presented with symptomatic hepatitis. In prospectively-studied patients, those who received commercial factor VIII had generally higher peak AST values (mean 22 times upper limit of normal, range 4·3–63) than those treated with NHS factor VIII (mean 13·8, range 2·8–40) or factor IX (mean 9·8, range 3·8–24).

The incubation period of acute NANB hepatitis (Fig 2) was related to the type of product infused, being shorter in patients treated with commercial factor VIII (mean 21 d, range 9–36) and factor IX (mean 18 d, range 7–26) than in patients treated with NHS factor VIII (mean 49 d, range 21–94). There was no apparent association between incubation periods and the dosage of factor VIII or IX. In patient 29, the incubation period for hepatitis B (the time interval between the first exposure infusion and the first detection of HBsAg) was prolonged at 134 d. The magnitude of previous blood product exposure did not appear to influence incubation periods or the severity of hepatitis. However, it was noticeable that the two patients treated with NHS factor VIII concentrate who did not develop NANB hepatitis (patients 20 and 21) had had the highest previous exposures in the NHS factor VIII treated group (300 and 1600 donor units, respectively), and were treated with relatively small doses of concentrate for their first exposure. In contrast, several of the patients who developed hepatitis after treatment with commercial factor VIII concentrates had previously been exposed to more than 300 donor units of NHS blood products.

Rates of progression to chronic hepatitis (i.e. evidence of continued AST abnormality 6 months after the onset of the acute attack) in patients who received no further treatment with blood products during the 6 month period after their first exposure courses were: 4/5 patients treated with commercial factor VIII, 2/5 patients treated with NHS factor VIII, and 1/3 patients treated with factor IX. Patient 29 was excluded from this analysis. All but one of the nine patients who received further treatment after their first exposure courses had an abnormal AST at 6 months.

Table I. Patient characteristics, treatment and outcome in prospectively-studied patients

Patient	Sex	Age (yr)	Product	Batch	Dose (u/kg)	Previous exposure (donor units)	Incubation period (d)	Peak AST (times upper limit of normal)	Symptoms*
1	M	63	Com VIII	A	860	500	10	5·3	S
2	M	53	Com VIII	B+C	670	800	9	63	SJ
3	F	29	Com VIII	D+E	34	190	18	23	SJ
4	M	77	Com VIII	F	15	300	34	8	SJ
5	M	23	Com VIII	G	11	500	35	19	O
6	F	34	Com VIII, ISG	H	781	5	36	29	SJ
7	M	21	Com VIII, ISG	I	164	200‡	20	7·4	O
8	M	19	Com VIII, ISG	I	26	1000‡	19	4·3	O
9	M	25	Com VIII, ISG	I	23	1000‡	9	39	SJ
10	M	4 mo	NHS VIII	J	300	Nil	28	2·8	O
11	M	13	NHS VIII	K+L	216	200‡	94	10	O
12	M	4	NHS VIII	M	210	Nil	42	3·1	O
13	M	55	NHS VIII	N	180	200‡	65	5·7	O
14	F	10	NHS VIII	O	149	35‡	58	2·5	O
15	M	8 mo	NHS VIII	P	110	Nil	48	18	O
16	M	27 mo	NHS VIII	Q	19	6	34	4·1	O
17	M	50	NHS VIII	R	16	100‡	21	24	S
18	M	42	NHS VIII	R	15	200‡	48	28	S
19	M	15	NHS VIII	S	9	200‡	50	40	SJ
20	F	34	NHS VIII	T	18	300	No hepatitis		
21	M	20	NHS VIII	U	8	1600	No hepatitis		
22	F	40	NHS Cryo		84 (70 donor units)	Nil	No hepatitis		
23	F	34	NHS Cryo		84 (70 donor units)	Nil	No hepatitis		
24	M	20	NHS Cryo		32 (35 donor units)	Nil	No hepatitis		
16	M	19 mo	NHS Cryo		30 (6 donor units)	Nil	No hepatitis		
25	M	6	NHS Cryo		12 (5 donor units)	Nil	No hepatitis		
26	M	19	NHS IX	a	339	20	18	24	O
27	M	16	NHS IX	b	102	Nil	26	3·8	O
28	M	22 mo	NHS IX	c+d	74	Nil	21	2·8	O
29†	M	52	NHS IX	e+f	34	Nil	7	8·5	S
30	F	38	NHS IX, ISG	g	89	10	No hepatitis		

* S/O indicates presence/absence of symptoms. J indicates the presence of clinical jaundice.

† Patient 29 also developed hepatitis B. Details shown refer to attack of NANB hepatitis.

‡ Patients treated with blood products (cryoprecipitate exclusively) in the 6 month period before their 'first exposure' infusion.

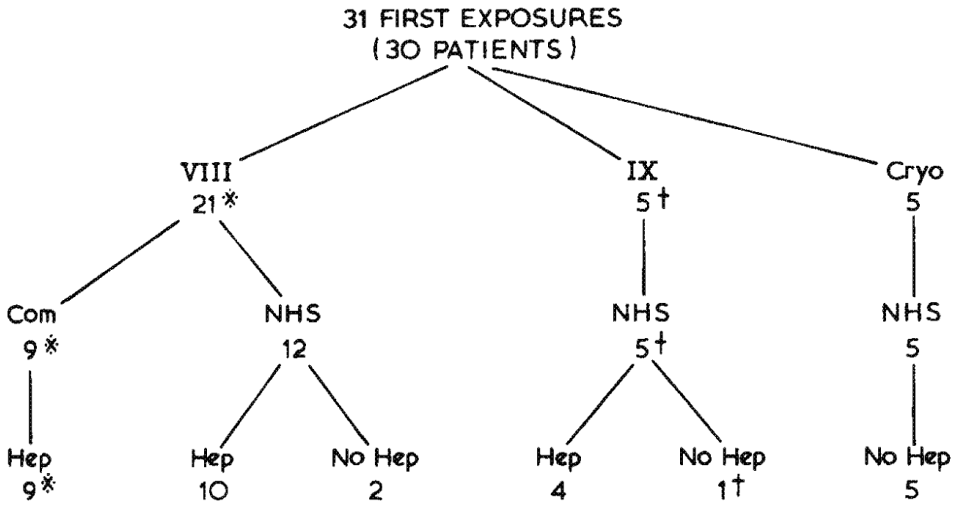

Fig 1. Outcome in prospectively studied patients. * Includes four patients treated with ISG. † Includes one patient treated with ISG.

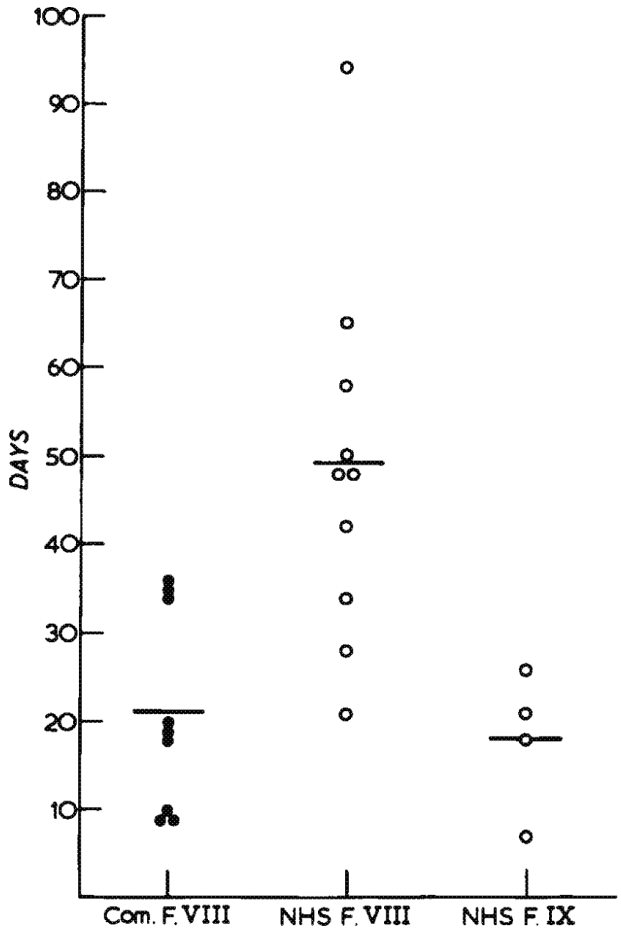

Fig 2. Incubation periods of acute non-A, non-B hepatitis after a first exposure to clotting factor concentrates. Horizontal bars indicate mean values.

ISG-treated patients

No adverse effects were observed after infusions of ISG. All patients treated with factor VIII and ISG developed NANB hepatitis and administration of ISG did not appear to prolong incubation periods, reduce peak AST values, or attenuate the rate of progression to chronicity. Of the three patients who received no further treatment with blood products in the 6 month period after their first exposure to concentrate (patients 6, 8 and 9) only one (patient 6) had normal liver function at 6 months. The single patient treated with factor IX and ISG (patient 30) did not develop hepatitis. However, an infusion of the same batch of factor IX (13 640 u, 164 u/kg) given without ISG to another patient, not included in this study because he had been previously exposed to factor IX concentrate, was followed by symptomatic hepatitis with a 16 d incubation period which resolved after 2 months.

DISCUSSION

In the U.S.A. the incidence of post-infusion NANB hepatitis after treatment with non-pooled commercial blood products averages about 28%, compared with 7% after products obtained from volunteers (Hollinger *et al*, 1981). In the U.K. the incidence of 2–4% after volunteer products (Medical Research Council Working Party, 1974; Collins *et al*, 1983) suggests that the prevalence of a carrier state amongst donors may be less than in the U.S.A. Even on the basis of lower attack rates reported in the U.K., e.g. a 2·4% incidence after a mean exposure to 7·24 units of blood products (Collins *et al*, 1983), it is probable that a susceptible patient exposed to more than about 300 donor units of blood products will develop post-infusion NANB hepatitis. Since clotting factor concentrates are usually prepared from pools of at least 1500 donor plasmas, it is not surprising that the overall attack rate following a first exposure to these products should approach 100%, whether they are of volunteer or commercial origin. Similar findings have been reported by others (Fletcher *et al*, 1983). Previously reported lower attack rates in comparable groups of patients (Kasper & Kipnis, 1972; Norkrans *et al*, 1981) are probably mainly attributable to reliance on symptoms rather than biochemical screening to detect hepatitis. In this study, even in prospectively monitored patients, the incidence of symptomatic hepatitis in concentrate-treated patients was only 43%. It was noticeable that in retrospectively studied patients, where reliance was placed on the patient to report illness to his physician, the detected incidence was even lower at 12%. The absence of hepatitis amongst our cryoprecipitate-treated patients probably reflects their relatively low exposures—none received more than 70 donor units.

There is conflicting evidence concerning the efficacy of ISG in the prevention or attenuation of post-infusion NANB hepatitis, benefit having mainly been found where blood from commercial or military sources has been used (Hollinger *et al*, 1981). Treatment with ISG before rather than after transfusion may be advantageous (Knodell *et al*, 1976) as may be addition of ISG to transfused blood (Kikuchi & Tateda, 1980). High titre anti-HBs, premixed with factor IX concentrate deliberately contaminated with HBV, has been shown to render the concentrate non-infective (Tabor *et al*, 1980). Despite these observations, and our use of ISG either before exposure to factor VIII or pre-mixed with factor VIII, we found no evidence of

a protective effect of ISG against commercial factor VIII-induced NANB hepatitis. It seems most likely that the ISG contained insufficient quantities of neutralizing antibodies to render NANB hepatitis invoking agent(s) in the concentrates non-infective. This might be a consequence of high concentrations of infective agents in the concentrates, and/or an infrequent or low titre virus neutralizing immune responses after infection with such agents in the ISG donor popoulation.

The apparent protective effect of U.S.A.-derived commercial ISG against NHS factor IX-induced hepatitis in a single patient requires cautious interpretation, since the patient may have been resistant to the transmissible agent(s) present in the concentrate. The 100% attack rate seen in other patients makes this unlikely. It is possible that the batch of ISG used to treat this patient contained higher than average levels of neutralizing antibodies, or that the factor IX concentrate contained lesser amounts of the NANB agent(s) present in commercial factor VIII concentrates. A different interpretation, supported by the results of cross-challenge experiments in chimpanzees (Tsiquaye & Zuckerman, 1979; Bradley *et al*, 1980; Hollinger *et al*, 1980), could be that the two types of concentrate contained different transmissible agents, only those present in the factor IX being neutralizable by ISG. This would suggest the presence of at least one similar agent in U.S.A. commercial and U.K. volunteer donor populations. The longer incubation periods observed after NHS than commercial factor VIIII concentrates, and the apparent active immunity conferred by high previous blood product exposure against hepatitis induced by the former but not the latter products, might also be explained on the basis of their contents of different transmissible agents. It is also possible that dosage effects could account for both these differences, and the propensity of commercial factor VIII to cause more severe and longer lasting hepatitis. The delayed onset of hepatitis B in patient 29 suggests viral 'interference', a phenomenon which has been noted in chimpanzees (Brotman *et al*, 1983; Bradley *et al*, 1983).

Whether prepared from volunteer or commercial donor plasma, clotting factor concentrates carry a very high risk of acute NANB hepatitis in first exposure recipients. Even substantial previous exposure to other blood products may reduce this risk only marginally. A critical component of the evaluation of new products of possible reduced infectivity will be their assessment in such patients, and we hope that the data presented in this report will provide a useful basis for comparative studies. One problem of such studies will be patient accrual, since many patients with mild bleeding disorders, who in the past might have been considered suitable for therapy with concentrates, are now considered more appropriately treated with cryoprecipitate or desmopressin (DDAVP). Because of the high attack rates seen with current products, however, the benefits or lack of benefits of new products should become apparent from study of only small numbers of suitably selected patients. We have not found ISG to be of value in preventing or attenuating hepatitis caused by commercial factor VIII concentrates. Whether it can confer protection against factor IX-induced disease remains to be confirmed.

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