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MINIREVIEW

Human Immunoglobulins for Intravenous Use and Hepatitis C Viral Transmission†

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INTRODUCTION

On February 23, 1994, a U.S. Food and Drug Administration (FDA)-licensed immunoglobulin intravenous (IGIV) manufacturer initiated a worldwide withdrawal of two products following an accumulation of 14 reported cases of hepatitis in American and European patients receiving one of these products. By July 1994, this number had grown to 112 in the United States alone (5). A newly licensed version of the product in question has since been introduced following the incorporation of a solvent-detergent treatment in the manufacturing process. This most recent episode has brought to light some unresolved questions concerning the past and future safety of IGIV products with respect to viral transmission in general and hepatitis C virus (HCV) in particular.

HCV

HCV is a small enveloped virus containing ~10 kb of single-stranded RNA, with a genetic organization which bears similarities to viruses in the pestivirus and flavivirus families (46). Its potential existence was acknowledged in 1974 with the introduction of the term non-A, non-B hepatitis (NANB) virus (58). Between 1974 and 1989, when HCV was successfully cloned (14), NANB viruses were noted to account for approximately 25% of all acute viral hepatitis cases and 75 to 90% of all posttransfusion viral hepatitis cases. With the development of serologic tests for HCV, retrospective studies have determined that 50 to 80% of acute sporadic NANB hepatitis cases are caused by HCV, while approximately 90% of posttransfusion NANB hepatitis cases are caused by this agent (1, 37, 67, 74).

Prior to the institution of HCV antibody screening, approximately 150,000 cases of NANB hepatitis occurred in the United States annually, with an estimated 150 million carriers worldwide (40). The course of infection and clinical outcome appear to vary according to the underlying health status of the patient and the route of infection, though the latter is likely to be a correlate of infectious dose. Few studies concerning risk factors for infection have been published. Prior to the use of transaminase screening of blood donors and viral inactivation of coagulation factors, it was estimated that 2 to 20% of blood product transfusion recipients (13, 43), 50% of parenteral drug users, and 70 to 100% of hemophiliacs developed NANB hepatitis (40). By examining cases of NANB or HCV for risk factors, transfusion was identified among 6 to 50%, intravenous drug use was identified among 16%, and contact with an infected person was identified among 12% (13, 23, 44, 71). Through a large epidemiologic study in the United States, the Centers for Disease Control and Prevention (CDC) determined that 59% of HCV-infected patients had a history of parenteral exposure (34% injection drug use, 21% transfusion, and 5% occupational exposure), 6% had sexual or household contacts, and 28% were from a low socioeconomic level. Only 7% had no identifiable risk category (3). As noted below, the risk from transfusion has decreased more than 80% since donor screening for anti-HCV was instituted, leaving intravenous drug use as the major risk factor. Oral-fecal transmission does not seem to occur, and sexual transmission is debatable.

For an otherwise healthy individual, symptoms and signs of infection appear in 20 to 40% of cases between 5 and 10 weeks postinfection and include fatigue, jaundice, pale stools, and dark urine with a palpable liver. Hemophiliacs appear to have a more rapid onset of symptoms, 2 to 4 weeks. Seroconversion as judged by a first-generation enzyme-linked immunosorbent assay (ELISA) (c100-3 clone single antigen [amino acids 1569 to 1931] [Fig. 1]) typically occurred by 10 to 12 weeks postinoculation. The false-positive rate with these early tests was quite high (24). Second-generation, multiple-antigen (three recombinant antigens) enzyme immunoassays and radioimmunoblot (four recombinant antigens) assays are more sensitive and specific (Table 1), with the ability to detect seroconversion as early as 4 weeks postinfection. Infected patients may thus be seropositive prior to the onset of clinical symptoms (60, 78). The majority of patients eventually seroconvert by 3 to 6 months postinfection, although repeated testing of suspected (seronegative) cases may be advisable for at least 1 year.

Thirty percent of acute sporadic infections resolve rapidly as judged by the resolution of hepatomegaly and aminotransferase levels in sera. In these patients, seroreversion was noted to occur over a period of up to 5 years by using a first-generation ELISA, though seroreversions are rarely seen with second-generation tests. A long-term carrier state develops in 50 to 70% of these cases, with persistent viremia in 95% of these cases despite a vigorous antibody response (62, 75). Approximately 5% of these patients show histopathologic changes indicative of chronic active infection, with an additional 5% showing cirrhosis.

The outcome appears to be somewhat different in cases of posttransfusion HCV infection. Resolution occurs in about 50% of these cases, but chronic active infection develops in 30% of them, with 10% going on to cirrhosis. The remaining 10% show chronic persistent infection (3, 31).

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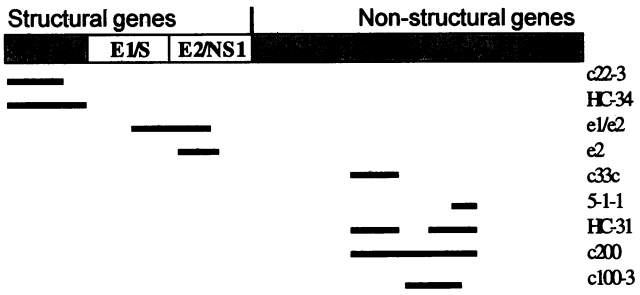

FIG. 1. Diagram of the HCV genome. The ranges of selected antigens are indicated below the genetic structure.

STUDIES OF VIRAL SAFETY

As with any regulated medicinal compound, manufacturers of IGIV products are required to provide data on both safety and efficacy in support of product license applications. These data are obtained through clinical trials in which liver function tests are routinely performed. It is accepted that IGIV products are not hepatotoxic, although immune-deficient patients may occasionally have transiently increased levels of transaminases (<2.5× upper limit of normal) following some infusions.

Manufacturers with products on the market during the late 1980s responded to the case reports of NANB viral transmission (see below) by publishing their relevant experiences over 6 or more months of monitored infusions (6, 59, 63, 65, 66). Cutter Biological published a prospective study of the safety of pH 4.25 IGIV (Gamimune) in 18 patients monitored for 14 to 26 months. Only minor elevations of alanine aminotransferase were seen, which is typical of the patient population studied (64). The Swiss Red Cross similarly reported on 68 patients monitored for 6 months (~204 infusions) who used lots of a product (Sandoglobulin) made by further fractionation of fractions I, II, and III produced by Baxter from plasma supplied by the American Red Cross. Again, no evidence of hepatitis was detected (27).

Other companies have similar data on file. For example, Armour Pharmaceuticals (Gammar I.V.) monitored over 1,100 clinical trial infusions, including a 4 3/4-year study involving 393 patient months of infusions (55). No significant liver enzyme elevations were seen, and no cases of hepatitis occurred. Such large manufacturers have distributed millions of grams of IGIV products, both before and after anti-HCV screening was utilized, with excellent viral safety.

Unfortunately, prospective safety studies did not predict the total experience of some manufacturers, such as the Scottish National Blood Transfusion Service (SNBTS). In response to a report by Lane et al. (34) concerning NANB cases and IGIV products, SNBTS began a prospective study of 16 hypogammaglobulinemic patients receiving regular infusions of its IGIV product. Each patient was monitored for 6 to 12 months, and during this time none showed evidence of hepatitis. Thirty-eight additional patients examined for 6 months or less were also free of hepatitis (35). A batch of identically prepared IGIV product manufactured later by the same laboratory was associated with viral transmission (82).

IGIV PRODUCTS AND VIRAL HEPATITIS

Clinical experience with IGIV products has been extensive, with millions of doses administered in the past 10 years. Early concerns about potentially increased risk from higher doses of IGIV products, relative to intramuscular immunoglobulins (200 to 1,000 mg/kg per infusion versus 100 mg/kg per injection), have not materialized (10, 34, 38). There are isolated exceptions to this general record of viral safety. Transmission of hepatitis B virus has occurred in the past through nonlicensed intramuscular preparations in the United States and small lots of immunoglobulin-containing products in Ireland, Germany, and South America (28, 47, 48, 56, 73). With the present screening of plasma and blood donors for hepatitis B surface antigen, a procedure recommended in 1977 by the World Health Organization, such a risk for commercial IGIV manufacturers has been greatly reduced.

Understanding and minimizing the risk for NANB/HCV has been more problematic. A review of the literature reveals 47 cases reported in association with five named IGIV products and 5 additional cases reported without identification of the product (Table 2). The clinical course of infection reported for these patients with common variable immunodeficiency, or hypogammaglobulinemia, appears to have been more fulminant than that for otherwise healthy patients. Seroconversion was not to be expected, although one patient is reported to have made immunoglobulin M (IgM) class antibody in response to the infection (26). Infection resulted in hepatic failure and death in 8 to 30% of these cases (10, 80) (Table 3). Approximately 25% of these patients went on to develop

TABLE 1. Detection specificities of HCV serologic tests

Antigen[a] (protein)	Detection by[b]:							
	Ortho HCV 1.0 ELISA	Abbott HCV EIA 1.0	Ortho HCV 2.0 ELISA	Abbott HCV EIA 2.0	Chiron RIBA HCV 2.0 SIA	Ortho PA (agglutination)	Chiron experimental ELISA	Abbott MATRIX HCV (experimental)
c22-3			X		X	X		
HC-34				X				X
e1/e2 (gp33/gp72)							X	
e2 (gp43)							X	
c33c					X			
HC-31				X				X
c200			X			X		
c100-3	X	X	X	X	X			
c100-3								X
5-1-1					X			

[a] The source for c22-3, c200, and the first c100-3 listed was *Saccharomyces cerevisiae*; the source for HC-34, c33c, HC-31, the second c100-3 listed, and 5-1-1 was *Escherichia coli*. The fusion protein for c22-3, c33c, c200, c100-3, and 5-1-1 was superoxide dismutase; the fusion protein for HC-34 and HC-31 was CMP-2-keto-3-deoxyoctulosonic acid synthetase.

[b] EIA, enzyme immunoassay; RIBA, radioimmunoblot assay; SIA, strip immunoblot assay.

TABLE 2. IGIV products and reported NANB (HCV) hepatitis cases

Brand (manufacturer and country)	No. of cases reported	Yr(s) of occurrence	Yr reported	Reference(s)
IGIV (British Blood Products Laboratory, United Kingdom)	12	1983	1984	11, 38, 39
Gammagard (Hyland, United States)	9	1984	1985	50, 51
	1	1990	1994	53
	>100	1993–1994	1994	5
Gammonativ (KabiVitrum, Sweden)	2	1981	1986	81
	1	1983	1986	81
	1	1985	1986	81
	16		1988	10
IGIV NOS[a]	1		1985	26
	3		1990	61
	1		1993	37, 40
Sandoglobulin (Sandoz, Switzerland)[b]	1	1983	1987	41
IVIgG (SNTBS, Scotland)	4	1987	1988	82, 83

[a] NOS, not otherwise specified.
[b] Patient received several units of blood 1 year previous to diagnosis of HCV infection.

cirrhosis, with an average of 45% developing chronic active disease (10, 51, 80, 81, 83).

Although these cases were dramatic, a perspective on their rarity is gained by a review of the literature on IGIV products overall, which indicates the existence of more than 30 brands produced in the United States, Japan, Italy, Austria, France, Spain, Norway, Germany, and Australia. The precise factors distinguishing the virus-contaminated lots from the majority of safe products could not be determined, which brought to light the corollary question of what it is about most IGIV preparations that makes them safe. Explanations have tended to focus on manufacturing controls, dedicated versus nondedicated equipment, cross-contamination, and small-scale or pilot manufacturing versus more stringently controlled full-scale production lots. Under pilot plant conditions, equipment may not be segregated, facilities are generally not separated, and rigorous validation of fraction separation may not be undertaken. Virus could presumably have been introduced during processing at a late step, or virus in the starting pooled plasma may have been inadequately removed or inactivated in these particular lots.

The assumption of inadequate manufacturing controls rests on a belief that fully licensed manufacturers, performing cold alcohol fractionation with dedicated equipment cleaned by validated sterilization procedures, did not and would not experience NANB contamination. Global regulations and recommendations concerning HCV antibody testing of plasma donors, intended to limit the potential for virus to enter the starting plasma pool, are evidence that the assumption was understood to be somewhat tenuous. The 1993 to 1994 cluster of HCV cases associated with a commercial-scale product brings all these assumptions into question again.

IGIV MANUFACTURING

IGIV products are manufactured from pooled plasma through a process of physicochemical separation of its various components. In 1946, Cohn and colleagues (15) described a simple and elegant scheme whereby the concentration of protein and ethanol, temperature, ionic strength, and pH were serially adjusted to precipitate reasonably discrete fractions. Cohn's method number 6 was particularly amenable to large-scale use. This method results in five major fractions: fraction I (fibrinogen), fractions II and III (gamma globulins), fraction IV (alpha and beta globulins), and fraction V (albumin).

As expected, differences in fractionation and purification processes between manufacturers exist and in aggregate lead to unique products, each of which satisfies the minimum uniform criteria set forth by the World Health Organization (4, 8). These differences begin with plasma collection (Fig. 2). Plasma may be obtained from volunteer or paid donors, the former generally involving recovery from whole blood and the latter involving plasmapheresis (source plasma). The seroprevalence of anti-HCV is in the range of 0.4 to 1.4% (71, 74, 79) for persons making volunteer donations, while approximately 6% (20) of those at plasmapheresis centers are seropositive. The latter figure varies widely according to factors such as the location and proportion of first-time versus repeat ("pedigreed") donors.

Variations of the original Cohn method (30, 52, 72), combined with more subtle adjustments in precipitation and filtration conditions, make it difficult to generalize about a cold alcohol procedure. The starting material for final manufacture of IGIV products can be fraction II (precipitate or powder), fraction III filtrate, or fraction GG of the Kistler-Nitschmann process. Various components may be removed prior to reaching the IGIV starting material. Typically, cryoprecipitate is taken off for factor VIII production, factor IX is removed for purification, and fraction I is discarded. Downstream processing may involve the use of one or more of the following: ion-exchange resins to remove IgA; polyethylene glycol and ultrafiltration to remove immune complexes, aggregates, and IgM; and partial digestion with proteolytic enzymes, low pH, and affinity chromatography to remove anti-A and anti-B

TABLE 3. Outcomes of HCV infections

Reference	No. of cases	Diagnoses[a]	Follow-up (yr)	No. of patients (%)			
				Died	Cirrhosis	CAH[b]	Recovered
Ochs et al. (51)	9	CVID, XLA, hyper-IgM	4	0 (0)	2 (22)	7 (78)	0 (0)
Webster and Lever (80)	12	Immunodeficiency	2	1 (8)	3 (24)	6 (50)	3 (24)
Weiland et al. (81)	4	2 normal, 2 hypogam	1–5	0 (0)	2 (50)	2 (50)	0 (0)
Björkander et al. (10)	16	13 CVID, 3 subclass deficient		5 (31)	3 (19)[c]	3 (19)	8 (50)
Williams et al. (83)	4	2 XLA, 1 CVID, 1 ulcerative colitis	1	0 (0)	0 (0)	1 (25)	3 (75)
Lockner et al. (41)	1	CVID	4	0 (0)	0 (0)	0 (0)	1 (100)

[a] CVID, common variable immunodeficiency; XLA, X-linked agammaglobulinemia; hypogam, hypogammaglobulinemia.
[b] CAH, chronic active hepatitis.
[c] Includes patients who died.

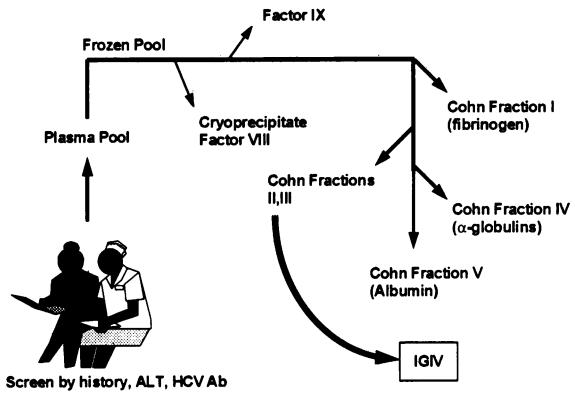

FIG. 2. Schematic diagram of fractionation process for IGIV products. Plasma donors are typically screened through a program which includes donor education, predonation questioning regarding health status and risk factors, and testing for alanine aminotransferase (ALT) elevation, hepatitis B surface antigen, and antibodies to human immunodeficiency virus types 1 and 2 and HCV (HCV Ab). Pooled units of test-negative plasma are stored frozen, with cryoprecipitate taken off for coagulation factor VIII during the thawing process. Chromatography may be used to collect coagulation factor IX, and Cohn fraction 1 may be precipitated before harvesting fraction 2 (± fraction 3) for further processing to immunoglobulin. HCV may partition differentially into these various fractions.

antibodies. Plasminogen, prekallikrein activators, fragments, and aggregates are typically minimized, though certain European IGIV products consist of $F(ab')_2$ fragments or contain significant amounts of the Fc fragment.

VIRUS INACTIVATION VERSUS REMOVAL

As with chemical and biological processes in general, the key to successful viral removal and/or killing is differential susceptibility to the process employed. Unlike the demonstrable inactivation and viral removal of human immunodeficiency virus by cold alcohol fractionation, this basic process alone does not sufficiently inactivate model viruses of HCV such as bovine viral diarrhea virus (33). The addition of harsh chemical processes is risky. For example, reduction and alkylation may have contributed positively to the excellent viral safety record of Cutter's original Gamimune (69) at the expense of questionable IgG Fc functionality.

S sulfonation and liquid heat treatment (pasteurization) have also been shown to be effective at inactivating members of the family *Flaviviridae* (49, 77), though dry heat treatment may not be adequate (12). Certain mild procedures which are currently used, such as ultrafiltration/diafiltration, polyethylene glycol precipitation, partial pepsin digestion, and low pH (29), are reported to be at least partially effective. However, there may be a threshold which can be exceeded for these techniques, as apparently happened at the SNBTS (82). The partitioning of HCV may contribute substantially to the safety of IGIV products (84). The >90% seropositivity rate for HCV among hemophiliacs is testimony to the partitioning of this virus with the starting materials for coagulation products (32, 76). With the development of exceptionally high purification techniques and viral inactivation steps for factors VIII and IX, this problem has been eliminated. Virus may also partition with fractions I through IV (84). The common practice of precipitating and discarding fraction I may reduce the amount of virus entering IGIV starting materials (Fig. 2). Fraction I was apparently not removed by several of the manufacturers whose products transmitted HCV in the past. Albumin, produced from fraction V, is pasteurized.

The effect of using DEAE ion-exchange chromatography has been debated, with various authors suggesting that it either decreases (59) or increases the partitioning of HCV with immunoglobulins (7). Although DEAE was used by Hyland and KabiVitrum in the preparation of their contaminated lots, it is generally thought that this would have been a neutral factor in that ion-exchange chromatography does not discriminate well between viruses and proteins.

CONTRIBUTION OF ANTIBODIES

The availability of serologic tests for HCV created a problem for IGIV manufacturers. Blood banks had already been utilizing alanine aminotransferase testing and hepatitis B core antibody (anti-HBc) testing to successfully identify 50 to 60% of HCV-infected potential donors, yet the risk for posttransfusion HCV infection remained fairly high for patients receiving 10 or more units (2, 13). Adding the HCV antibody screening test led to an 85% further reduction in the risk for transfusion recipients (21, 78).

IGIV products, meanwhile, had only rarely transmitted this virus, and prior to the initiation of HCV antibody screening, 90% or more of commercially available IGIV preparations contained HCV antibody, though this varied by the country of plasma origin (19, 61). The degree to which anti-HCV antibodies in the product contributed to its safety was unknown. Some data existed to suggest that IGIV or intramuscular immunoglobulin was at least partially effective as prophylaxis against NANB hepatitis (16, 17, 54, 57, 68, 70, 77), but this efficacy fell short of the experience with prophylaxis against hepatitis A and B viruses (72). With the experience of the blood banks making it clear that excluding HCV antibody-positive donors would reduce the number of viremic units entering the plasma pool, the question became one of balance. Was there more good in decreasing virus-positive units than detriment in removing antibodies from the pool? That is, could the blending of antibody-positive units in plasma pools help in any way to offset the potential for infection from virus which would likely enter these pools from donors in the window period prior to transaminase elevation and seroconversion?

Vigorous debate on this point ensued, with the FDA taking the position that antibody was potentially a critical factor in maintaining the safety of the product (22). Two studies suggested an inverse correlation between the presence of antibody by second-generation tests and the presence of viral RNA by PCR (18, 36). Such data would be consistent with the hypothesis that the virus formed complexes with antibody and was removed during purification, but no direct evidence exists to support this contention. To address the overall question experimentally, the FDA sponsored a test to observe the effect of removing anti-HCV antibodies, as detected by the c100-3 first-generation test, from a plasma pool. Not unexpectedly, the pool from 2,887 test-negative donors proved to be infectious in each of two recipient chimpanzees. This pool was then split and provided to seven manufacturers holding United States licenses, each of which processed its aliquot into IGIV products by its standard procedures. Three chimpanzees were then infused with all seven preparations at a total dose of 1,000 mg/kg. After 7 months of observation and testing, it was concluded that none showed evidence of infection (9, 25). It thus appeared that screened plasma still contributed infectious virus to the pool and that processing in some way removed and/or inactivated some amount of virus, despite the absence of those antibodies detected by the first-generation test.

TABLE 4. General chronology of the adoption of HCV antibody screening recommendations

Source[a]	Date of adoption of recommendations
Japan (Red Cross)	November 1989
Australia (NBTC)	February 1990
France (Ministry of Health)	March 1990
United States (FDA), whole blood and transfusable components	May 1990
Canada (Red Cross)	June 1990
The Netherlands	Late 1990
Switzerland	Late 1990
United Kingdom (NIBSC)	September 1991
United States (FDA), plasma for further manufacture	April 1992
Germany (BGA)	April 1992
European Community (CPMC)	January 1993

[a] NIBSC, National Institute for Biological Standards and Controls; BGA, Bundesgeshundheitsamt; NBTC, National Blood Transfusion Committee; CPMC, Committee on Proprietary Medicines and Controls.

Consistent with the advice of its Blood Products Advisory Committee, the FDA agreed in October 1991 to accept a draft guidance document prepared by United States manufacturers which recommended antibody screening of source plasma. In April 1992, the FDA followed other countries in formally issuing the recommendation that all source plasma donors be screened and positive units be excluded from pools. Since January 1993, all plasma collected worldwide for fractionation into human products derives from antibody-negative donors (Table 4). A few finished products still in the distribution pipeline, derived from antibody-positive pools, may continue to be sold for a short time. Whereas HCV transmission has not occurred except apparently in association with specific lots of Gammagard not treated with solvent-detergent, many observers feel that the contribution of anti-HCV antibodies to viral safety was less than imagined. The effect of utilizing current, more sensitive second-generation tests has not been studied. While it can be assumed that more sensitive screening tests result in the removal of additional anti-HCV antibody from the pool, such as potentially neutralizing anti-E1/E2, it is not yet clear what the impact of removal will be. It is possible that these additional antibodies provided some element of safety.

CONCLUSIONS

Techniques specifically designed to destroy viruses, such as solvent-detergent and/or liquid heat treatment (60°C for 10 h), have recently been introduced by several licensed manufacturers in the United States. Some have demonstrated the virucidal nature of their existing processes (42), while others continue to rely for the moment upon the excellent safety records of existing processes. The question of HCV risk with IGIV products will largely be answered when every manufacturer has demonstrated validated viral reduction steps in its process. While screening all patients who receive regular infusions of IGIV products for HCV infection is not recommended, screening those believed to be at risk because of particular exposures should follow the recent Centers for Disease Control and Prevention recommendations (5). It is important to recognize that the serologic testing of immunodeficient patients is not sufficient.

Nonenveloped viruses, such as parvoviruses, and as yet undetected viruses resistant to current methods of elimination may pose an incalculable risk for the future, but the issue more likely to be debated in coming years is the substitution of a viral detection test for HCV in place of antibody screening. That is, as is the case with hepatitis B virus, it can be envisioned that a highly sensitive HCV RNA detection test could be used along with alanine aminotransferase levels to screen potential donors, potentially allowing anti-HCV antibody back into the pool (45). Similar discussions concerning the continuing requirement to exclude anti-HBc-positive donors from plasma pools, an antibody which generally coexists with neutralizing anti-HB antibodies, have recently taken place in France.

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