The epidemiology of virus transmission by plasma derivatives: clinical studies verifying the lack of transmission of hepatitis B and C viruses and HIV type 1

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Therapeutic products made from human plasma provide life-saving relief to hundreds of thousands of patients each year. The efficient production of these derivatives requires that they be made from large pools of human plasma, often comprising as many as 60,000 donations each. This review will examine the viruses that can be transmitted by plasma and for which sensitive detection systems and virus-removal and -inactivation methods are available.

The exclusion of plasma units that test positive for certain viral markers ensures that almost all units entering the pool are free of viruses. Each plasma donation is screened by using sensitive immunoassays to detect the presence of hepatitis B virus (HBV), hepatitis C virus (HCV), and HIV types 1 and 2 (HIV-1/2). Manufacturing steps for plasma products have been shown to remove or inactivate these viruses.

Nevertheless, plasma units containing these viruses can enter the pool when a donor is in the window period of the disease, which is the short period during which the virus is present in the blood before the screening tests have become positive. At present, the screening of small pools of plasma ("minipools") by using nucleic acid amplification assays such as polymerase chain reaction (PCR) is being introduced widely on an investigational basis, and it will result in a shortening of the window periods of these viruses. Improvements in nucleic acid amplification assays in the future to make the screening of individual plasma units cost-effective could result in the elimination of nearly all window-period donations containing these viruses.

Virus-removal and -inactivation procedures during the manufacture of plasma derivatives ensure that any residual HBV, HCV, HIV-1, or HIV-2 in the pool does not result in an infectious product. Among apparently healthy donors of source plasma (plasma collected by plasmapheresis) with no serologic markers of these viruses, there are approximately 54 HBV window-period donations, 36 HCV window-period donations, and 1.5 HIV-1 window-period donations per million donations.[1] (The rates of window-period donations for recovered plasma [plasma obtained from whole-blood donations] are 29, 33.5, and 1.5 for HBV, HCV, and HIV-1, respectively.[1]) This means that as many as 1 in every 10 plasma pools (each containing plasma from up to 60,000 donations) could contain an HIV-infected unit, and every pool of this size could contain 2 HBV-infected window-period units and 2 HCV-infected window-period units. Thus, many plasma derivatives rely on the removal of viruses by viral-inactivation procedures or the manufacturing process in general to prevent transmission.

HBV was recognized as a risk to pooled plasma derivatives long before the licensure in 1973 of sensitive third-generation assays to detect the hepatitis B surface antigen (HBsAg) in donor blood and before testing became required by the Food and Drug Administration (FDA) in 1975. The transmission of hepatitis in 1943 to 70,000 United States troops in North Africa as a result of the administration of a yellow fever vaccine diluted with human serum underlined the potential risk. (This infection was shown to be due to HBV when stored sera were tested in the 1970s with the serologic tests that had become available by then.) By 1975, the application of sensitive tests for HBsAg to exclude HBV-infected units left 90 percent of the remaining cases of posttransfusion hepatitis classified as "non-A,non-B hepatitis,"[2] which was

ABBREVIATIONS: AHF = antihemophilic factor; FDA = Food and Drug Administration; F IX = factor IX complex; HBsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HIV-1/2 = HIV types 1 and 2; IGIM = intramuscularly administered immune globulin; IGIV = intravenously administered immune globulin; PCR = polymerase chain reaction; PPF = plasma protein fraction.

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Presented at Food and Drug Administration Blood Products Advisory Committee meetings, June 20, 1997, and December 11, 1998.

Received for publication November 2, 1998; revision received April 2, 1999, and accepted April 6, 1999.

TRANSFUSION 1999;39:1160-1168.

shown mostly to be HCV when assays to detect anti-HCV became available (described in 1988; licensed in 1990).

The clinical aspects of AIDS were first recognized between 1981 and 1983; initially, the viral etiology of this disease was hotly debated. By 1984, however, the viral etiology of AIDS and the risk to pooled plasma were clear, particularly in connection with the high prevalence of the virus among hemophiliacs. HIV was identified in 1984, and a sensitive assay to detect anti-HIV was licensed in 1985.

Before the application of virus-inactivation procedures to most plasma derivatives (which required the development of methods to stabilize those derivatives that were too unstable to withstand heating), the concept of high-risk and low-risk products developed. The concept of low-risk products was based on the lack of transmission of HBV by albumin and immune globulin in clinical experience as well as in volunteer studies (see below). This concept was extended to HIV and HCV, initially on the basis of the epidemiologic similarities to HBV infection and later on the basis of the absence of transmission of these viruses by the low-risk products. Conversely, products such as antihemophilic factor (AHF) and factor IX complex (F IX) were considered high risk; efforts were made to reduce their level of risk by screening donors with a history of relevant risk factors and by serologic tests, but these products continued to transmit infection to hemophiliacs until methods were developed that would stabilize them enough to permit inactivation of viruses by heat and other methods.

One can now divide plasma derivatives into three risk categories: 1) inactivated products with a long history of use, including albumin and plasma protein fraction (PPF); 2) inactivated products with a shorter history of use of the inactivated forms, including AHF and F IX; and 3) immune globulins, which had a long history of safe use without added inactivation procedures (although most have undergone inactivation procedures in recent years).

ALBUMIN

Albumin has the longest record of safety of any plasma derivative. Heat stabilization of albumin was developed during World War II to improve the physical stability of the product for military shipment to desert areas. It was soon recognized that this stability permitted the application of heat to inactivate an infectious agent present in some donor plasma; this agent was known to be present because of the transmission of hepatitis (now known to have been HBV) to recipients of the donors' plasma.

Albumin and HBV

There has been no transmission of HBV by albumin during more than 50 years of widespread clinical use, even in the years before HBsAg-positive blood could be eliminated by serologic screening. The infusion of albumin to millions of patients during these years provides strong evidence of the efficacy of virus removal and inactivation provided in the manufacturing process.

In a study conducted in 1952 by Paine and Janeway,[3] 237 albumin recipients (receiving albumin from 92 production lots) were prospectively followed to determine the prevalence of hepatitis. The high prevalence of hepatitis in the donor population, combined with the effects of pooling, led to an expectation that jaundice would be observed in 39 percent of recipients. Of the 33 recipients who received only albumin, however, none developed jaundice. Of the 204 who had received other blood products as well as albumin, only 2 (1%) developed jaundice.[3]

After World War II, studies were conducted in human volunteers to evaluate the efficacy of heat inactivation of the hepatitis agent(s) (at that time still unidentified) that were known to be present in pooled plasma. Gellis et al.[4] prepared a plasma sample, which was known to transmit hepatitis, as a 20-percent solution in albumin. The solution was heated at 60°C for 10 hours. Of 10 volunteers intramuscularly injected with 10 mL of the heated material, none developed hepatitis; 3 of 5 injected with the same volume of unheated material developed hepatitis.

Murray and Diefenbach[5] and colleagues[6,7] (and also reported in part by Pennell[8]) conducted a series of studies between 1951 and 1953 evaluating various ways to inactivate hepatitis agent(s) in plasma, including heat treatment. They used an experimental plasma pool made of plasma from donors known to transmit hepatitis, shown in titration studies to contain $10^{7.5}$ infectious doses of the virus per mL (in other words, 10^{-7} to 10^{-8} mL [less than one-ten-millionth of an mL] could theoretically transmit the infection). Later studies showed that the virus was HBV and that the pool had an HBsAg titer of 1:100 by radioimmunoassay. In the first study,[5] heating albumin prepared from this pool at 60°C for 2 or 4 hours did not inactivate the virus (Table 1).

In the second study, heating albumin prepared from this pool at 60°C for 10 hours prevented the transmission of hepatitis after the subcutaneous inoculation of 3 mL[6,8] or the intravenous inoculation of 100 mL.[7] Alt hough unheated albumin did not transmit hepatitis when only 3 mL was injected, 100 mL of unheated albumin or 1 mL of unheated plasma from the pool did transmit hepatitis (Table 1). A similar experiment showed that the heating (at 60°C for 10 hours) of a product called stable plasma protein solution (SPPS), a precursor of today's PPF (made from the same infectious plasma pool), prevented the transmission of hepatitis; in contrast, recipients of 1 mL of unheated stable plasma protein solution made from this pool became infected after subcutaneous inoculation[8] (Table 1). These studies showed that the manufacturing process for albumin removed most of the hepatitis infectivity, even when an enormous amount of virus was present in the starting pool, and that heating this product at 60°C for 10 hours removed any residual infectivity.

TABLE 1. Inactivation of HBV in albumin as determined by studies[5-8] of volunteer recipients

Experiment	Inoculum	Treatment	Volume	Hepatitis in recipients
1	Infected plasma pool*	60°C × 2 hours plasma	1 mL	4/10
		60°C × 4 hours plasma	1 mL	5/10
		Unheated plasma	1 mL	2/5
2	Infected plasma pool*	60°C × 10 hours albumin†	3 mL	0/10
		60°C × 10 hours albumin	100 mL	0/10‡
		Unheated albumin	3 mL	0/10
		Unheated albumin	100 mL	2/10
		Unheated plasma	1 mL	5/10
3	Infected plasma pool*	60°C × 10 hours SPPS§	1 mL	0/10
		Unheated SPPS	1 mL	2/5

* $10^{7.5}$ infectious doses per mL; HBsAg titer of 1:100 by radioimmunoassay.
† Albumin prepared from the infected plasma pool.
‡ Recipients of 100 mL of albumin heated at 60°C for 10 hours were reported as 10 subjects in the original report[8]; however, 15 subjects were reported in a later publication.[7]
§ SPPS = stable plasma protein solution, a forerunner of PPF.

(It is of interest to note that Roderick Murray, the principal investigator in these studies, later [1955-1972] was the first and only director of the Division of Biologics Standards, National Institutes of Health, a forerunner of today's Center for Biologics Evaluation and Research, FDA. One of the other senior investigators in these studies, John W. Oliphant, was committed to the concept that hepatitis could be eliminated from plasma by inactivation with betapropiolactone or ultraviolet irradiation. When these methods failed and three volunteers died of fulminant hepatitis, Dr. Oliphant committed suicide.)

It is important that heat inactivation be applied after the manufacturing process has removed much of the viral load, because heat alone is not capable of removing all HBV infectivity, at least in unprocessed plasma. Soulier et al.[9] studied the effect of heat on HBV infectivity in the course of preparing an experimental heat-inactivated HBV vaccine. Serum containing HBV with an HBsAg titer of 1:16 or 1:1024 (assay method not reported) was heated at 60°C for 10 hours. (It is almost certain that the serum must have been diluted to permit heating without denaturing the serum, but this was not clearly stated.) The higher-titer material transmitted hepatitis to six of seven recipients of four subcutaneous injections of 2 mL each (0/4 recipients of the lower-titer material developed hepatitis). Similar findings were reported in chimpanzee studies; heating a serum pool containing HBV, diluted 1-in-1000 in phosphate-buffered saline, at 60°C for 10 hours, did not prevent transmission of HBV.[10] The authors of that study estimated that infectivity had been reduced to 1/10,000th of the original level, as determined by the lengthening of the incubation period of HBV infections observed in the inoculated chimpanzees.

The importance of heating the final container rather than heating at an earlier stage of the manufacturing process and the existence of a greater margin of safety for albumin than for PPF were shown during a 1973 outbreak of HBV that was associated with two lots of PPF from one manufacturer.[11,12] Among recipients of those two lots, HBsAg became detectable in 5 percent. Investigation revealed that the heating process of that manufacturer, which involved heating the final product in bulk, had failed to heat adequately a small amount of PPF sequestered in a "sampling neck" of the bulk processing container. Albumin made by the same manufacturer from the same donor base (described as "similar donor sources") did not transmit HBV.[12] This was the only instance during the approximately 40-year history of PPF use in which hepatitis transmission has been known to occur. Since 1977, all PPF, as well as all albumin, has been heated in the final container, and no further episodes of transmission have occurred.

Albumin and HCV

Albumin has never transmitted non-A,non-B hepatitis, either before or after the availability of serologic screening of plasma for anti-HCV. The susceptibility of HCV to inactivation by the heating of up to 10^4 infectious doses per mL at 60°C for 10 hours has been shown in chimpanzee studies.[13,14]

Albumin and HIV

There has never been a case of the transmission of HIV by albumin, even by albumin made from plasma collected before screening of donors for HIV became possible. It has been shown that HIV is exquisitely sensitive to inactivation by heating: 10^5 HIV infectious doses per mL (in tissue culture medium with 10% fetal calf serum) can be eliminated by heating at 60°C for 10 minutes, as determined by lymphocyte cultures[15] (>$10^{4.6}$ reduction has been shown by others[16]). Thus, heating HIV for only one-sixtieth as long as albumin (10 min vs. 10 hours) can inactivate at least 1 log more virus than the maximum concentration reported in plasma of infected persons (10^4 infectious doses/mL[17]) and 2 logs more than the concentration usually found in plasma or peripheral blood monocytes (10^3 infectious doses/mL[18-21]). (These infectivity titers are lower than the PCR titers of the same or similar samples. PCR titers of HIV in plasma have been reported to be as high as 10^7 viral nucleic acid copies per mL, although 10^8 copies per mL can occasionally be detected as a result of test variation [Hewlett I, written communication, June 1998].)

IMMUNE GLOBULIN PRODUCTS

Clinical experience and volunteer studies led to the justifiable conclusion that immune globulin products made from plasma screened by current US-licensed screening tests

and fractionated by US-licensed methods will not transmit HBV, HCV, or HIV. However, the transmission of HCV in one outbreak (see below) made it clear that immune globulin products can be considered safe only if safety is reevaluated with any change in the methods of donor selection or of manufacture. The reasons that immune globulin products are safe from virus transmission are somewhat uncertain; it is not known to what extent the fractionation process removes or inactivates viruses and to what extent the presence of neutralizing antibodies in the plasma of the many donors in the pool contributes to inactivating viruses from other donors or to partitioning the virus in the form of antibody–virus complexes.

Immune globulin products include the intramuscularly administered immune globulin (IGIM), the intravenously administered immune globulin (IGIV), and several products with specialized intramuscular uses: Rh_0 (D) immune globulin, hepatitis B immune globulin, etc. The cold ethanol fractionation method of manufacturing immune globulin products (Cohn-Oncley fractionation, consisting of Cohn Method 6 and Oncley Method 9), the only method used to make IGIM and most other immune globulin products licensed in the United States, results in a product that is safe from virus transmission. (All currently licensed immune globulin products, including IGIV, are made by methods that include some form of alcohol fractionation, except for one immune globulin product for intravenous use made by anion exchange chromatography. Like all intravenous immune globulin products, the latter is subjected to virus-inactivation procedures.) Methods such as anion exchange chromatography have been shown to leave transmissible HCV, resulting in transmission to recipients in outbreaks in Germany and in Ireland when not subjected to virus-inactivation procedures.[22-25]

The Cohn-Oncley fractionation process itself has been shown to reduce by a factor of 4.7×10^4 the amount of HCV RNA in the globulin fraction derived from an anti-HCV-positive plasma pool.[26] The HCV RNA detected in IGIM lots made by the Cohn-Oncley fractionation method in the years before the screening of individual plasma units for anti-HCV could have consisted of fragmented noninfectious HCV RNA, or it may have been intact virus, which would indicate that anti-HCV in the plasma pool contributed to the safety record of IGIM with regard to HCV. The protective role of antibody in the pool[27] was confirmed when lots of IGIV produced from plasma that did not contain detectable anti-HCV transmitted HCV; this incident, called the "Gammagard incident," is described below.

Immune globulin and HBV

IGIM has never transmitted HBV in the more than 20 years since the introduction of HBsAg screening of donors. Only one episode of HBV transmission was ever documented during the years before sensitive third-generation screening of donors for HBsAg (see below); however, it is possible that subclinical cases occurred but were not recognized without serologic screening tests. IGIM did not transmit hepatitis B in volunteer studies conducted in the 1950s (see below), and later testing of stored serum samples from the volunteer studies revealed no HBsAg in recipients.

In the one outbreak resulting from IGIM made from plasma collected before third-generation HBsAg screening, an IGIM lot with detectable HBsAg transmitted HBV infection.[28] This lot had much lower titers of antibody to HBsAg (anti-HBs) than would be expected, presumably because of the complexing of anti-HBs by HBsAg. There is further evidence that such unrecognized immune complex formation in IGIM might have been more widespread before 1973, when third-generation screening for HBsAg came into use, and that it clearly did not occur after 1973. In 1967, almost all lots of IGIM had anti-HBs titers <1:100, whereas, beginning in 1979, all lots had titers ≥1:100.[29,30] Beginning in 1972, all plasma units pooled to make IGIM were HBsAg negative. Separation of immune complexes found in IGIM lots from before 1972 indicated that the HBsAg in the pools prior to 1972 ended up in HBsAg–anti-HBs immune complexes, and such complexes were found in 78 percent of IGIM lots produced between 1962 and 1971.[30] Lots of IGIM produced between 1973 and 1977 had no complexes and no HBsAg.[30] Thus, lots made from plasma screened for HBsAg with third-generation assays were negative for HBsAg even when efforts were made to disrupt immune complexes.

The inoculation of volunteers subcutaneously with 2 mL of IGIM made by Cohn Method 6 and Oncley Method 9 from a plasma pool containing $10^{7.5}$ infectious doses of HBV (the same pool used in the albumin studies described above) showed that these methods of preparing IGIM removed the HBV infectivity.[31] Of 10 volunteers inoculated with the IGIM preparation, none developed hepatitis (and none had developed serologic evidence of infection, as shown by serologic testing when the stored sera were studied in later years); 2 of 5 inoculated with 1 mL of plasma from the original pool developed hepatitis.

It is not clear what role was played by plasma pool anti-HBs in rendering the IGIM noninfectious in these volunteer studies, because the pool of infectious plasma had an excess of HBsAg (HBsAg titer 1:100 by radioimmunoassay). It is possible that anti-HBs in the pool could have reduced the virus load to a noninfectious level, as the infectivity titer of plasma containing HBV is generally 1/100th to 1/1,000th that of the HBsAg titer.[32]

Usually, however, the fractionation process clears most of the free HBV and most of the HBsAg in the plasma pool, and it concentrates the IgG antibodies (including anti-HBs) in the fraction that would be used to manufacture IGIM. Any remaining HBV or HBsAg would be neutralized by the excess anti-HBs in that product. From the volunteer study just cited, it appears that, even in the presence of excess HBsAg in the plasma pool, the resulting IGIM would be ren-

dered noninfectious, presumably as a result of the neutralization of intact HBV to a level below that required to transmit infection. However, such an IGIM preparation without detectable anti-HBs would not have been made since the introduction of current procedures for HBsAg screening.

Immune globulin and HCV

Although many lots of IGIM made before 1994 were found in later studies to have had detectable HCV RNA,[33,34] HCV has never been transmitted by IGIM. It has been shown that plasma pools made from donations screened by second-generation multi-antigen assays for anti-HCV had no detectable HCV RNA by PCR, whereas 90 percent of plasma pools made before the introduction of any anti-HCV screening did have HCV RNA.[35] The role of plasma pool anti-HCV in reducing the risk that products made from that pool would transmit HCV is illustrated by the finding that experimental Cohn-Oncley fractionation of an anti-HCV-positive plasma pool resulted in a greater (4.7 $\log_{10}$) reduction in HCV RNA level than fractionation of an anti-HCV-negative pool (3.5 $\log_{10}$ reduction).[36] The safety of IGIM with regard to HCV has been documented in several follow-up studies of immune-deficient patients who have received weekly injections of IGIM for up to 3 years.[37,38] In one study, 12 patients received IGIM and 12 others received an experimental IGIV made from plasma from the same donors; all recipients of the IGIV acquired non-A,non-B hepatitis, while none of the IGIM group did so.[37] In another study, there was no transmission of HCV to 27 patients by lots of IGIM prepared from plasma from a similar group of donors as those whose plasma had been used to prepare the lots of IGIV that had transmitted HCV to 16 of 77 patients.[38]

As an extra safety precaution, IGIM licensed in the United States (with the exceptions noted below) has been tested by the FDA in the final product for HCV RNA by PCR since 1995. All lots released have had no detectable HCV RNA (Yu MW, written communication, July 1998). IGIM made by manufacturers who have introduced validated virus-inactivation and -removal procedures for this product is released from the requirement for final product testing for HCV RNA.

In 1993, there was an outbreak of HCV infection in recipients of an intravenous immune globulin (IGIV; Gammagard, Baxter Healthcare, Glendale, CA) made by one manufacturer; this outbreak has been known by the trade name of the product as the "Gammagard incident." There was no transmission of HCV by any IGIV made by any other US-licensed manufacturer, although several incidents were reported in Europe with IGIV preparations not marketed in the United States.[37-40] In a study of one cohort in the Gammagard incident, 23 (11%) of 210 recipients of Gammagard developed HCV infection, as compared to 0 of 52 recipients of other IGIVs studied.[41] In an analysis at one center, nine Gammagard lots were implicated out of 43 lots administered that had been made from plasma screened by second-generation (multi-antigen) screening tests for anti-HCV, which had recently been introduced.[41] Thus, in retrospect, 9 (21%) of 43 "at-risk" lots were implicated. Infections occurred in recipients of HCV RNA-positive lots; no infections occurred in those who received only HCV RNA-negative lots.[41] The likelihood of infection was directly proportional to the amount of HCV RNA[41,42] and the amount of IGIV received; the infection rate reached 29 percent in those who received the greatest quantity of HCV RNA[41] and 24 percent in those who received the greatest amount of Gammagard made from plasma that had been screened by second-generation assays for anti-HCV.[41]

In a series of elegant studies, Yu and colleagues[42] showed that the advances from unscreened plasma to screening of donor plasma units with first-generation (single-antigen) screening tests for anti-HCV to screening with the more sensitive, second-generation (multi-antigen) tests progressively removed much of the anti-HCV that had been bound in complexes to HCV after pooling, leaving unbound virus in the lots made after the more sensitive tests were introduced. Apparently, the same effect did not occur in IGIV made by other manufacturers or in IGIM, probably because of differences in the manufacturing methods that provided a greater margin of safety for virus removal in the latter products. (Similar studies were also reported by others.[43])

Methods were developed to stabilize IGIV to permit virus inactivation by heating; other validated virus-inactivation methods are used by some manufacturers of IGIV, such as solvent detergent treatment, treatment with pepsin at pH 4.0, and treatment with immobilized trypsin (Yu MW, written communication, April 1998). The application of virus-inactivation procedures to IGIV became universal by 1995. There has been no transmission of HCV by IGIV since 1994.

Immune globulin and HIV

HIV has not been transmitted by immune globulin products manufactured under US licenses. There were no seroconversions to anti-HIV in recipients of anti-HIV-positive lots of IGIM, IGIV, or hepatitis B immune globulin during the years 1982 through 1985, when HIV was already present in the plasma donor population but when anti-HIV screening of blood and plasma was not yet available.[44,45] Other specialty immune globulin products, such as tetanus immune globulin, $Rh_0(D)$ immune globulin, rabies immune globulin, and varicella zoster immune globulin, also have never been reported to transmit HIV. The entire process of manufacturing immune globulin products by Cohn Method 6 and Oncley Method 9 can remove $>10^{15}$ infectious doses of HIV per mL,[44,46,47] whereas plasma from an infected individual has no more than 10^4 infectious doses per mL[17] and usually no more than 10^3 infectious doses per mL.[18-21] Fur-

thermore, it has been shown that HIV could not be cultured from 38 lots of IGIM, IGIV, and hepatitis B immune globulin with detectable anti-HIV titers made before screening for anti-HIV was available,[45] although it has been notoriously difficult to culture HIV from plasma derivatives (even from lots of AHF known to transmit HIV infection[48] (Hewlett I, written communication, January 1998]).

AHF AND F IX

AHF and F IX were considered high-risk products for the transmission of viruses until methods were found to stabilize them so that virus-inactivation methods that involved heating could be used without damaging the coagulant activity of the products. Before the introduction of virus inactivation, most recipients of AHF and F IX eventually became infected with HBV; when HCV was recognized (initially as "non-A,non-B hepatitis"), it was found that most hemophiliacs also acquired HCV infection, with a high percentage developing chronic infection. The first methods of inactivating HBV experimentally in AHF were reported from Germany in 1980[49] and 1981.[50] These reports were not widely recognized because they were published in German-language journals and were not initially presented at any international meetings. Once the reports were recognized, inactivation of HBV in AHF and F IX was not immediately implemented in the United States, because of concern that the heating process reduced the concentration of the active components in the products; strong opposition on this basis was expressed by the major hemophilia patient organization at that time.[51] When virus-inactivation procedures were applied to AHF beginning in early 1983, the primary purpose was to inactivate HBV, and the methods had been validated and approved for inactivation of HBV.

At that time, many investigators believed that AIDS was not an infectious disease. At the meeting of the FDA's Blood Products Advisory Committee on February 7, 1983, one committee member stated, "I don't think there is a shred of evidence that this is transmitted by blood as of today."[52(p80)] An informal poll during a break in the meeting showed that most committee members did not think AIDS was caused by an infectious agent (the poll and its outcome are recollections of the author and of one other FDA official). Thus, the first virus-inactivation procedures were applied to AHF and F IX at a time when many experts on blood collection and processing did not think that AIDS was an infectious disease. By late 1983 and early 1984, however, the infectious nature of AIDS was generally accepted and the risk of HIV transmission by AHF and F IX had been recognized. These advances provided a stimulus for the further development and application of methods to inactivate viruses in AHF and F IX.

By 1985, all new lots of AHF and F IX had been subjected to some virus-inactivation procedure. Despite the failure of some of the early methods to inactivate HIV successfully, by 1987, there were suitable virus-inactivation procedures in the manufacturing process for all licensed AHF and F IX products. Today, each manufacturer subjects these products to more than one process that has been shown to be effective in either removing or inactivating viruses. These processes can consist of heating an aqueous solution of the final product or an intermediate preparation at 60°C for 10 hours or heating a lyophilized final product at various temperatures and times, such as 80°C for 72 hours.

Other processes involve treating an intermediate solution with a solvent such as tri-n-butyl phosphate and a nonionic detergent (Tween 80, deoxycholate, or Triton X-100). In addition, many steps in the purification processes have been shown to remove viruses. Overall, most manufacturing processes (including the inactivation steps) have been shown to inactivate or remove $>10^9$ HIV-infectious doses per mL (Lynch T, written communication, April 1998). In addition, the process of lyophilization itself can inactivate from $10^{1.5}$ to 10^4 HIV-infectious doses per mL in AHF concentrate.[53,54]

AHF, F IX, and HBV

There has been no transmission of HBV by US-licensed AHF or F IX since the application of effective virus-inactivation procedures by all manufacturers in 1987. This has been documented by studies with serial serologic tests of previously untreated hemophiliac patients who received only virus-inactivated AHF or F IX. The absence of transmission has been due not only to virus inactivation but also to the fact that those procedures have been applied to products made from plasma that was screened for HBsAg.

AHF, F IX, and HCV

All lots of AHF and F IX made after the introduction of plasma testing for anti-HCV and the introduction of virus inactivation have been found to be negative for HCV RNA.[55] No transmission of HCV by these products has occurred. In a prospective survey conducted by the National Hemophilia Foundation in collaboration with the Centers for Disease Control and Prevention and the FDA during a 3-year period from mid-1993 to mid-1996, no confirmed seroconversions to anti-HCV were found in patients at any of 71 hemophilia treatment centers in the United States (Final Technical Report, FDA contract #223-93-1005, 1996). These 71 treatment centers represented half of the 142 hemophilia treatment centers nationwide and about 35 percent of all US hemophiliacs.

There have been several studies that confirm the absence of HCV from AHF. In one study, HCV RNA could not be detected in a lot of AHF that had been derived in part from five plasma pools containing HCV RNA.[56] In another study, HCV RNA was not detected in any of 38 lots of AHF manufactured from 1992 to 1993, after the onset of testing

plasma donations for anti-HCV, although it was detected in 76 of 183 lots of AHF made between 1976 and 1991.[55] In addition, it has been shown that detectable HCV RNA has been eliminated from pools of recovered plasma as a result of the screening of donors for anti-HCV with second-generation multi-antigen assays.[35]

AHF, F IX, and HIV

There have been no seroconversions to anti-HIV in hemophiliacs who have received only AHF or F IX that has been made from screened plasma and that has been subjected to adequate virus-inactivation steps.[57] In a survey of all hemophilia treatment centers in the United States conducted from 1987 to 1990 by the National Hemophilia Foundation, no seroconversions to anti-HIV were found in patients who had received only AHF or F IX that had been subjected to virus-inactivation procedures licensed after 1987.[57] In the surveillance study conducted by the National Hemophilia Foundation, Centers for Disease Control and Prevention, and FDA, described above, no confirmed seroconversions to anti-HIV were found in 71 hemophilia treatment centers between 1993 to 1996 (Final Technical Report, FDA contract #223-93-1005, 1996).

SUMMARY

During the past 50 years, most US-licensed plasma derivatives have maintained an impressive record of not transmitting HBV, HCV, or HIV. Albumin (50-year history) has never transmitted these viruses. PPF (40-year history) transmitted HBV on only one occasion, which was associated with a design flaw in one manufacturing plant. IGIM has never transmitted any of these viruses since the requirement of sensitive serologic screening tests for HBV (24 years). IGIV (17-year history) transmitted HCV in only one outbreak involving the product of one manufacturer. Even AHF and F IX have not transmitted these viruses since effective virus-inactivation processes in manufacturing were developed. In summary, there has been no transmission of HBV, HCV, or HIV by US-licensed plasma derivatives since the introduction of effective virus-inactivation procedures. This means, essentially, that there has been no transmission of these viruses since the end of 1987; the sole exception is IGIV, by which there has been no transmission since 1994.

ACKNOWLEDGMENT

The author thanks John Finlayson, PhD, for invaluable advice on plasma derivatives.

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