The Process: New Methods of Purification and Viral Safety

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From the transmission of hepatitis C virus by gammaglobulins in 1994 to the emergence of new viruses and concern over prions, intravenous immunoglobulin (IGIV) manufacturers have continued to address safety issues and respond to changing needs. New IGIV products not only provide superior antiviral safety, but also show advances in product purity and manufacturing processes. Several manufacturers have also addressed the concern over prion transmission. The sum of the processes used have collectively ensured continuous product safety. Newer products will be further differentiated by their tolerability and efficacy profiles.

Key Words: process, purification, viral safety, product safety, prions.

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The prophylactic and therapeutic use of human immunoglobulin G (IgG) has a history spanning almost 50 years, and IgG products have generally had a good safety record. Gammaglobulin was first administered in 1956 by intramuscular injection. Intramuscular gammaglobulins became an effective first-line therapy for some patients, particularly those with hypogammaglobulinemia. The injections were painful, however, and gammaglobulin therapy remained limited not only by patient discomfort, but also by the amount that could be given and inconsistent absorption. Because these early preparations contained gammaglobulin aggregates capable of fixing complement, intravenous administration was not possible. Subsequent changes in manufacturing led to greater purity of intact immunoglobulins. Human intravenous immunoglobulin (IGIV), the form now used clinically, was introduced first in Europe and Australia in the 1960s and later in the United States in the 1970s.

Except for a couple of sporadic and poorly documented reports of hepatitis B virus (HBV) transmission by IGIV,[1, 2] concern over viral transmission with these products first attracted widespread attention in 1993–1994, when more than 125 patients became infected with hepatitis C virus (HCV) after receiving Gammagard (Baxter Healthcare Corp., Westlake Village, CA) and Polygam (American Red Cross) IGIV products.[3] Considerable effort was expended to discover the reasons for the unexpected infection. Researchers discovered that the manufacturing process at the time probably involved inadequate antiviral treatment. This was especially critical when more sensitive testing resulted in the removal of plasma containing hepatitis C antibodies that were most likely providing protection. As a result of this instance, manufacturers reviewed their manufacturing processes, and many implemented additional antiviral steps.

A further problem with early IGIV preparations was a lack of gammaglobulin purity and function. Because of enzymatic and chemical modifications during manufacture, first-generation IGIV products had a variety of functional disadvantages, such as abnormal distribution of IgG subclasses, some loss of Fc function, short half-lives, and decreased opsonic activity. In the second generation of products, some of these problems were corrected. This also resulted in an IgG subclass distribution that was closer to that of healthy individuals. The newest products have a normal subclass distribution, with a high purity of intact IgG molecules.

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Newer products also have better side-effect and safety profiles due to improved manufacturing processes and more effective steps to ensure protection from pathogens.

Current Antiviral Steps in IgG Manufacture

Assurance of safety has greatly improved since the first gammaglobulin products were manufactured; numerous steps and methods are now incorporated in the process (Figure 1). These methods can be classified generally as screening, viral inactivation, and viral removal.[4]

Reducing the potential for pathogen transmission begins with screening, both before and after the plasma donation is collected. All potential blood donors are screened thoroughly by medical history, physical examination, and any behavior or other factors that might put them at risk for blood-transmissible infections such as hepatitis or human immunodeficiency virus (HIV). After the plasma is collected, it undergoes extensive testing for antibodies to HBV, HCV, and HIV. Further testing is performed for the liver enzyme alanine aminotransferase, a nonspecific marker for hepatitis.

In addition, beginning in 2001, plasma has been tested by polymerase chain reaction for detection of nucleic acids—an indication of continuing infection. Polymerase chain reaction testing is used to examine the plasma for HCV and possibly, depending on the manufacturer, for HBV, hepatitis A virus (HAV), HIV, and/or human parvovirus B19. As an additional safety step, the plasma of first-time donors is held, without processing, until they return to donate a second time. This holding procedure protects the plasma pool against donations that were made after a viral infection but before serologic conversion and viral detection.

After the plasma is screened, it is entered into the manufacturing process, where the second stage of viral safety assurance begins. Although the fractionation process itself can inactivate and remove viruses and viral fragments, most manufacturers have added one or more additional treatments to inactivate or remove viruses that may remain undetected despite the rigorous screening process. It should be noted that inactivation does not eliminate the virus, but destroys the ability of the virus to replicate and infect patients.[5] Since some of the inactivation techniques also have the potential to adversely affect immunoglobulins, the manufacturers must select methods that will preserve the biologic integrity of the IgG molecule. The balance between viral inactivation and IgG preservation can be a very delicate one.

Manufacturers can choose from a variety of inactivation techniques. Because the different techniques can affect the biologic function of the IgG molecule, the methods of viral inactivation may account for some of the differences among IGIV products in terms of side effects and efficacy (Table 1). Of the methods listed, some

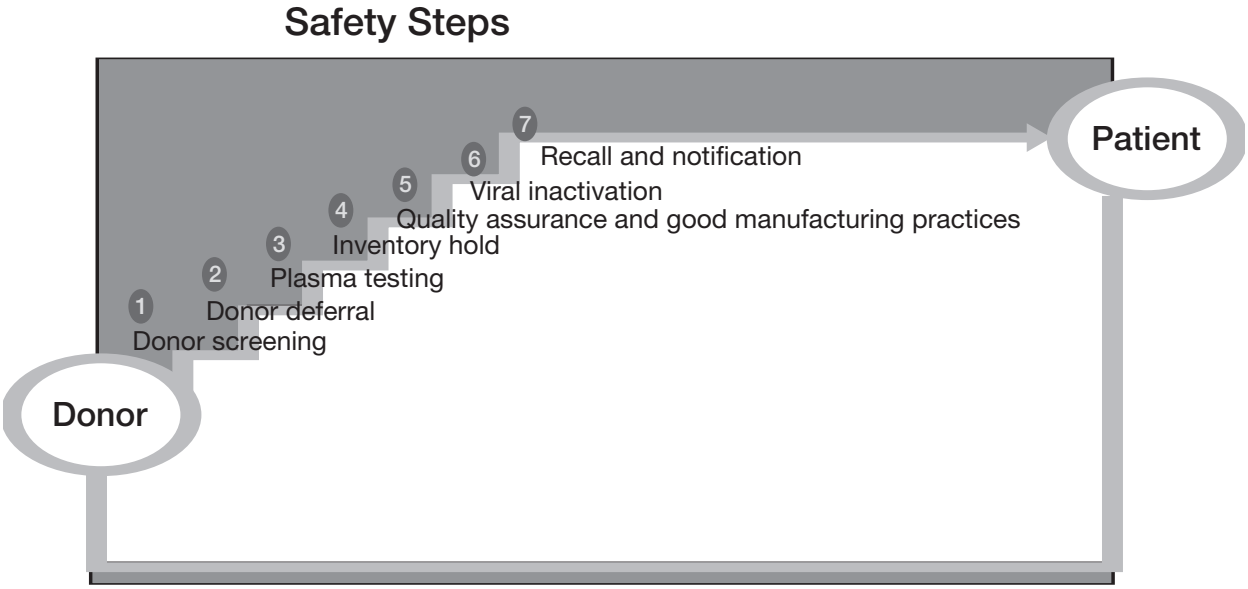

Figure 1. The intravenous immunoglobulin process from donor to patient.

Table 1. Methods Used by Manufacturers of Intravenous Immunoglobulin to Inactivate Viruses

Type	Method
Physical	Dry heat
	Heat treatment of freeze-dried products
	Pasteurization
Chemical, enzymatic	Solvent/detergents (e.g., Tween in tri-*N*-butylphosphate)
	Incubation at low pH
	Incubation at low pH + enzymatic treatment (pepsin digestion)
	Chemical alteration of viral RNA to impede replication
	Methylene blue (photosensitizer requiring activation by white light)
	Psoralens (photosensitizer requiring activation by long-wave ultraviolet light
	Riboflavin (photosensitizer requiring activation by white light)
	Caprylate (plant-derived fatty acid)

Table 2. Differences in Virus Inactivation Methods Among Intravenous Immunoglobulin Products

Product (manufacturer)	Solvent-Detergent	Pasteurization Treatment	Low pH (4.25)	Pepsin, pH 4	Trypsin	Caprylate
Carimune NF (ZLB Behring)				X		
Flebogamma (Grifols USA)		X				
Gammagard S/D (Baxter Healthcare Corp.)	X					
Gammar-P IV (ZLB Behring)		X				
Gamunex (Talecris Biotherapeutics)			X			X
Iveegam EN (Immuno US)					X	
Octagam (Octapharma USA)	X		X			

are preferable to others. Low pH treatment destroys viruses and, at the same time, stabilizes IgG molecules in the preferred monomer form in solution.[6–8] Caprylate quickly and effectively inactivates a wide spectrum of viruses and does not appear to have a significant impact on the concentration or function of the IgG molecule.

Viruses lacking a lipid envelope, such as human parvovirus 19 and HAV, are more difficult to inactivate than enveloped viruses. Although pasteurization has a greater ability to inactivate these viruses than many of the other methods, it can also affect the integrity of the IgG molecule. Therefore, for these viruses (and any similar virus not yet detected), the preferred method is simply to remove them from the plasma.[5] Although the fractionation process itself can remove viruses by partitioning, to remove additional viruses, manufacturers can choose precipitation, filtration (depth filtration, nanofiltration), or chromatography. Of note, caprylate, which is used to inactivate viruses, can also be used to precipitate non–IgG proteins from the plasma.

The highest level of viral safety is probably obtained by using complementary, independent steps because some methods are more effective against particular viruses than others. Not all manufacturers use the same steps or a single strategy to eliminate viruses (Table 2). Chromatography and filtration or nanofiltration are effective methods for removing most pathogens; however, if the protein and virus are similar in size, these methods alone will not eliminate the pathogen. This stresses the importance of multiple, independent, and complementary steps.[4, 5, 9]

The value of viral elimination steps is best confirmed using a wide range of test or model viruses to ensure that the inactivation and removal steps are effective for both known viruses and other potential emerging pathogens. The tests measure the extent to which a particular method reduces known titers of virus added to test samples of plasma. In these validation tests, a sample of plasma is spiked with the test virus or viral model and subjected to a scaled-down production process that includes all the viral reduction steps. Then, as a final step, the sample is tested for viral load.

Also, the virus inactivation and removal steps must be robust with respect to variations in processing parameters. The IGIV manufacturers alter production parameters (e.g., temperature,

pH) beyond a fixed manufacturing point to the operating limits or just outside the limits, and evaluate virus reduction. This way, they can ensure that virus inactivation and removal are consistent and effective within production operating ranges.

Safety Considerations

Because intravenous immunoglobulins are derived from human plasma, they are potentially susceptible to contamination by a variety of blood-borne pathogens; consequently, patients receiving and clinicians administering IGIV are concerned about disease transmission. Excellent screening methods and viral elimination steps in processing IGIVs help alleviate these fears. However, as demonstrated by the discovery of new viruses (e.g., hantavirus, the coronavirus that causes severe acute respiratory syndrome [commonly known as SARS], West Nile virus, monkeypox virus, and bird flu virus), not all viruses that are potentially pathogenic for humans have been identified. Therefore, IGIV manufacturers must remain vigilant.

Another concern is the potential for prion transmission through blood or plasma products. Prions are extremely stable, infectious-like proteins that are responsible for a variety of diseases, often referred to as spongiform encephalopathies.[10] Not all prions are bad, as "normal" prions exist in the brain. Although the role of these normal prions is not fully elucidated, they are felt to be involved in the memory process.[11] Prions are unique in that although they are relatively simple proteins, they are able to replicate much like a bacteria or virus. These aberrant prions ultimately concentrate in the brain where they replicate and destroy brain tissue. The term "spongiform" is used because the brain in such cases is often filled with holes and resembles a sponge. Although rare in humans, these diseases are common in animals. One of the most common forms, found in sheep and goats, is called scrapie. In mule and deer it is referred to as chronic wasting disease. When it occurs in humans, it is called Creutzfeldt-Jakob disease (CJD). In all cases of spongiform encephalopathies, there is no treatment, and the disease ultimately results in death.

There are two forms of CJD in humans: classic and variant. Classic CJD can be inherited or occur spontaneously. It has also been transmitted (from infected donors) through dura matter or corneal transplants, as well as pituitary

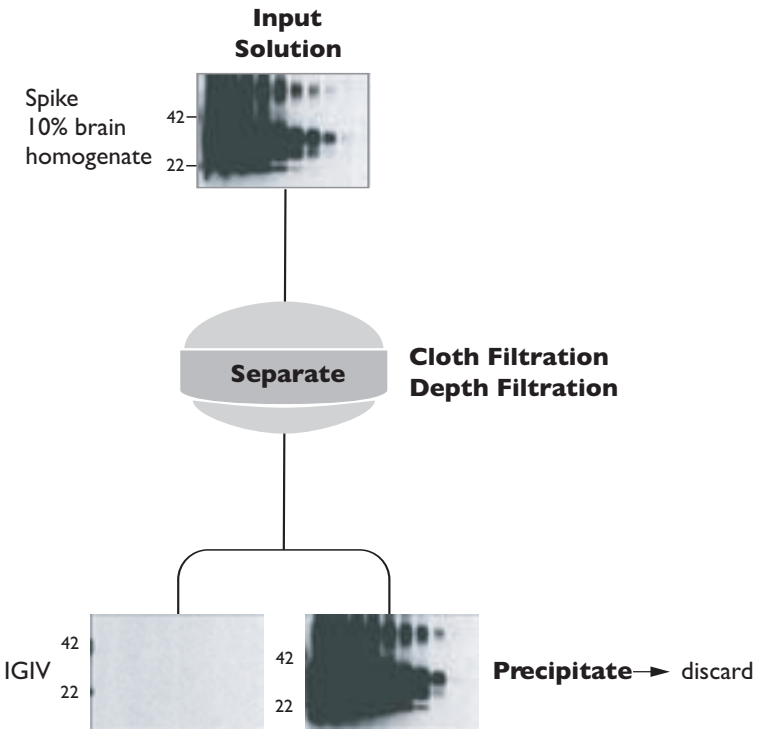

Figure 2. Methods used by intravenous immunoglobulin manufacturers to remove prions.

growth hormone injections. The occurrence of CJD is rare and estimated as approximately 1/1,000,000 individuals.[12] It is not felt to be a risk in terms of transmission through blood or blood products.

In 1995, a new prion disorder, termed variant CJD (vCJD), appeared in the United Kingdom. It was apparently transmitted by meat from cattle with bovine spongiform encephalopathy (commonly known as mad cow disease). Many of the more than 100 human victims were young, which suggests a shorter incubation period than classic CJD. Because of the shorter incubation period of vCJD and the fact that there might be lymph node involvement, it is felt that there is more of a risk of transmission of vCJD through blood or blood products. This has received even more attention as a result of several reports in the United Kingdom of possible vCJD transmission through blood transfusions.[13] In the United States, as a precaution, restrictions were placed on blood donors who had lived in the United Kingdom for 6 months or longer, and current Food and Drug Administration guidelines require withdrawal of any batch of plasma derivatives if a pool donor is subsequently found to have vCJD. Manufacturers of IGIVs are again revising their protocols to ensure that their products do not transmit the causative prion because older purification methods may not protect against prions.

Until recently, no practical method was available to test for markers of infectious CJD agents in plasma. However, a new, highly sensitive Western blot assay enables manufacturers to ensure that their IGIV products are not contaminated with prions and that safety measures are as effective as possible (Figure 2). Briefly, this in vitro assay permits markers of transmissible spongiform encephalopathies to be detected and quantified rapidly. To test the effectiveness of prion removal steps, plasma samples are spiked with protease-resistant prion protein, a marker for transmissible spongiform encephalopathies. The samples are subsequently assayed with the Western blot technique after each manufacturing step. To date, there has been no evidence of prion transmission through IGIV products, and the risk is thought to be very small. Currently, three IGIV products (Carimune NF [ZLB Behring, Berne, Switzerland], Gamunex [Talecris Biotherapeutics, Clayton, NC], and Octagam [Octapharma USA, Centreville, VA]) have data to support a 3.5-log or greater reduction of prions during the production process.[14–16]

Conclusion

From the transmission of HCV by gammaglobulins in 1994 to the emergence of new viruses or concern over prions, IGIV manufacturers have continued to address safety issues and respond to changing needs. New IGIV products not only provide superior antiviral safety, but also show advances in product purity and manufacturing processes. Several manufacturers have also addressed the concern over prion transmission. The sum of the processes used have collectively ensured continuous product safety; newer products will be further differentiated by their tolerability and efficacy profiles.

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