Transfusion–Transmitted Viruses

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ABBREVIATIONS: AABB = American Association of Blood Banks; ALT = alanine aminotransferase; AMT = aminomethyl trimethyl psoralen; anti-HBc = anti-hepatitis B core antigen; BPV = bovine papillomavirus; BSE = bovine spongiform encephalopathy; CDC = Centers for Disease Control; CJD = Crutzfeldt-Jakob disease; CMV = cytomegalovirus; DMMB = dimethymethylene blue; EBV = Epstein-Barr virus; FDA = Food and Drug Administration; FRALE = fangible anchor length effectors; GBV-C = hepatitis GB virus; GGT = gamma glutamyl transferase; HAV = hepatitis A virus; HbsAg = hepatitis B surface antigen; HBV = hepatitis B virus; HCV = hepatitis C virus; HGV = hepatitis G virus; HHV = human herpes virus; HIV = human immunodeficiency virus; HPV = human papillomavirus; HTLV = human T-cell lymphotropic virus I and II; NAT = nucleic acid testing; NIH = National Institutes of Health; REDS = Retrovirus Epidemiology Donor Study; S-D = solvent-detergent process; SD-FFP = solvent-detergent fresh frozen plasma; SEN-V = SEN virus; TNBP = tri (*n*-butyl) phosphate; TTV = transfusion-transmitted virus; UVA = ultraviolet A; vCJD = variant Crutzfeldt-Jakob disease; WNV = West Nile virus.

INDEX TERMS: blood testing; transfusion.

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The number and variety of viruses that have been proven to be transmitted by the transfusion of blood and blood products continue to increase. Among the viruses that are familiar to the clinical laboratory scientist are those for which the blood supply is currently tested: human immunodeficiency virus human immunodeficiency virus 1/2 (HIV 1/2), human T-cell lymphotropic virus (HTLV) I and II, hepatitis C virus (HCV), hepatitis B virus (HBV), and, in some cases, cytomegalovirus (CMV). The transmission of West Nile virus (WNV) by both transfusion and transplantation has recently emerged as a concern. Donor testing for WNV was implemented as soon as a test for its detection was approved in the summer of 2003. Additionally, the testing of donor units for hepatitis A virus (HAV) and parvovirus B19 is under review and is likely to be implemented in the near future. Donors who have either resided or traveled in the United Kingdom (UK)or continental Europe for an accumulation of three months between the years 1980 and 1996, or injected bovine insulin from the UK, are deferred from blood donation in the United States (U.S.) because they are considered to be at risk for variant Crutzfeldt-Jakob Disease (vCJD), a form of spongiform encephalopathy that may be transfusion-transmissible.

Several other viruses are either transfusion-transmitted or suspected of being so. SEN virus (SEN-V) has recently been investigated in transfusion-associated hepatitis. Transfusion-transmitted virus (TTV), an aptly named viral agent, is prevalent in the donor population and may be associated with chronic alanine aminotransferase (ALT) elevation in some individuals. Transfusion has been implicated in the trans-

mission of Epstein-Barr virus (EBV) in a case of possible transfusion-transmitted infectious mononucleosis. EBV has also been associated with posttransfusion hepatitis. Several human herpes viruses (HHV-6, HHV-7 and HHV-8) have been studied as to possible parenteral transmission. A virus known variously as hepatitis GB virus (GBV-C) and hepatitis G virus (HGV) has also been determined to be transfusion-transmitted, although it does not appear to cause any disease state and is not associated with hepatitis, as previously thought. Bovine papillomavirus (BPV) has been shown to be transfusion-transmitted in cattle, raising questions about human papillomavirus (HPV).

The number and variety of the viruses that have been associated with blood transfusion require the immunohematologist to be familiar with the viruses and the methods of detection and/or inactivation that may be employed to protect the transfused patient from viral transmission. All of the viruses listed above will be reviewed in this article, along with their clinical significance, testing being performed to detect them, and treatments of blood components to inactivate them.

FAMILIAR TRANSFUSION-TRANSMITTED VIRUSES

U.S. donors are currently screened for infection with HCV, HBV, HIV, and HTLV-I and –II, and in some instances, CMV. HCV is a single-stranded RNA virus of the Flaviviridae family. HCV transmission in the U.S. appears to be primarily parenteral, with sexual transmission a less efficient form of transmission. Blood transfusion accounted for a large number of cases identified prior to the implementation of donor screening for the virus. Greater than 90% of the cases of non-A, non-B hepatitis caused by blood transfusion were due to the transmission of HCV prior to 1990.[1] Between 1990 and 1998, anti-HCV testing was used alone to detect infected donors. As a result of this testing, the incidence of transfusion-transmitted HCV was reduced to approximately 1 in 103,000.[2] In 1998, nucleic acid testing (NAT) for HCV RNA was implemented to screen all blood donors in the U.S. Since that time, both anti-HCV and NAT testing for HCV RNA have been used in the U.S. and the current risk of transmission per unit of blood donated has been estimated to be 1 in 1,600,000.[3,4]

HBV is a virus of the family Hepadnaviridae and, unlike HCV or HAV, has a DNA genome. This virus, too, is primarily transmitted sexually and parenterally; however, it can also be transmitted from mother to child. Donor testing for HBV includes hepatitis B surface antigen (HBsAg) and anti-hepatitis B core antigen (anti-HBc). Alanine aminotransferase (ALT) which detects acute hepatic inflammation regardless of cause, was previously required as a surrogate test for hepatitis virus or liver disease. The ALT is no longer required by either the Food and Drug Administration (FDA) or American Association of Blood Banks (AABB) regulations for blood donor testing. Today, the ALT is still performed by many manufacturers of plasma products, primarily as a surrogate test for hepatitis viruses other than HBV or HCV. In 1996, the Retrovirus Epidemiology Donor Study (REDS) estimated that 1 in 63,000 donations in the U.S. may transmit HBV to the recipients because the donor, although HBV positive, was in the pre-HBsAg positive 'window period' when the donation was made. Therefore, even with donor testing for HBsAg and anti-HBc in place, some transfusion-transmission of HBV was unavoidable.[3,5] Source plasma manufacturers have recently implemented NAT testing for HBV DNA, as have blood donor facilities in Germany and Japan.[6,7] Although there is considerable discussion regarding the need to do so, it seems likely that HBV DNA NAT testing will be adopted in the U.S. soon. Examples of the conflicting reports include a study by Otake and Nishioka in Japan that showed the detection of HBV DNA by NAT method in seven units that were tested as a part of minipools of 50 donors. All seven of these donor units were negative for HBsAg, HBcAg, anti-HBs, and anti-HBc by the Auszyme II enzyme immunoassay method.[6] However, the U.S. studies cited by Sacher and coworkers indicated that HBV is present in very small amounts during the window period.[8] Studies in the U.S. that have evaluated several HBsAg methods against several HBV DNA NAT methods appears to show that, while currently FDA licensed methods for NAT testing will detect infected donors that are not detected by currently licensed HBsAg methods, there are HBsAg methods under FDA review (though not yet licensed) that are as good as the NAT methods available.[9,10]

HIV is a member of the Lentivirus subfamily of the Retroviridae. As with HCV, the transmission is primarily sexual and parenteral, although maternal-child transmission is also possible. The implementation of HIV antibody testing of donors in 1985 decreased the number of transfusion-transmitted cases from 714 reported in 1984 to about five cases per year from 1985 to 1990. In 1996 the risk per donor was calculated to be 1 in 493,000.[2,7] In 1996, HIV p24 antigen testing was added to anti-HIV testing, further reducing the risk of transmission to 1 in 641,000.[11] The cumulative number of HIV cases attributable to transfusion-transmission prior to June of 1999 was 8,793.[12] Of these cases, only 40 were traced to blood transfusions given after

the implementation of HIV antibody testing in March of 1985. The few cases due to screening failure at this time resulted from donations from infected individuals with viremia that were obtained prior to the development of detectable antibody responses, the so-called 'window period'. The development and subsequent implementation of p24 testing reduced the 'window period' from 45 days in 1990 to about 16 days, due to the ability to detect circulating viral antigen earlier than detection of antibody.[13,14] However, a few cases of transfusion-transmitted HIV still occurred due to donation during the 'window period'.

HIV type-1 RNA has been detected in the U.S. blood donor population by NAT since 1998, further reducing the window period to about 11 days.[3,15] Testing for HIV-1 RNA has replaced testing for p24 in U.S. donor population. Due to cost concerns, pools of plasma from 16 to 24 donations, rather than individual donor samples, are currently NAT tested for HIV-1 RNA. A case reported in 2003 documented the transmission of HIV in a unit of red cells that had screened negative for HIV RNA using the minipool NAT screening currently employed.[16] Further reductions in the window period may be possible by testing each unit for HIV-1 RNA separately. As a result of using minipool NAT testing in conjunction with anti-HIV 1/2 testing, the incidence of transfusion-transmitted HIV has been reduced to approximately 1:1,800,000.[17] It is not possible, however, to detect every infected unit due to decreased viral load in some individuals, viral mutation, and the failure of some infected individuals to form antibody.

HTLV-I and HTLV-II were the first of the human retroviruses to be identified as transfusion-transmitted. These viruses have been implicated in T-cell leukemias, polymyositis, arthritis, and myelopathies and are also transmitted through sexual, IV drug abuse, and perinatal mother to child methods. The prevalence of antibodies to HTLV was shown to be 0.025% in the U.S. donor population in 1988, the year donor testing was introduced for antibodies to HTLV-I.[18] Blood containing HTLV that has been stored for more than 14 days, as well as non-cellular blood components, appears to be non-infectious.[11] Using current tests that detect antibody specific to both HTLV-I and HTLV-II, the risk of HTLV transmission is estimated by Schreiber and colleagues to be approximately 1 in 641,000 units.[2] However, serious clinical disease is seen in approximately 5% of individuals infected with HTLV, indicating that the actual risk of disease is considerably lower.

CMV is a DNA virus of the Herpesviridae family that is easily transmitted by transfusion, but is also spread through the saliva and the semen. It is usually not significant in immunocompetent individuals, but can be of serious consequence, and sometimes fatal, in neonates, infants, and immunosuppressed patients. Organ and tissue transplant patients are at risk not only for transfusion-transmitted, but also for transplantation-transmitted CMV. No routine screening of the blood donor population is currently employed. The virus is leukocyte-associated and leukoreduction of blood products is effective in reducing transmission. The presence of less than 10^7 leukocytes per unit achieved through leukoreduction makes CMV transmission less likely.[19] Transfusion-transmitted CMV is currently controlled in susceptible populations through either leukoreduction of the blood product or the use of products negative for anti-CMV. The transmission of CMV when these types of products are used can occur, however, with rates reported as high as 2%.[20] One cause for such transmission may be plasma viremia during the first four weeks of infection (the 'window period'). Drew and colleagues, in a study of a CMV DNA PCR assay, showed that plasma CMV DNA was detected in two instances before the development of CMV antibody.[21] There are conflicting reports, though, about the efficacy of testing for CMV DNA. A large cross-sectional study of U.S. blood donors showed that CMV DNA was not detected in any of 514 samples that were negative for anti-CMV, nor was CMV DNA detected in any of 70 samples that had discrepant CMV serology results.[22] The authors of that study concluded that the addition of CMV DNA PCR to donor screening would not increase detection of CMV positive donors over screening for anti-CMV.

OTHER VIRULENT TRANSFUSION-TRANSMITTED VIRUSES

Several other virulent viruses are known to be transmitted through transfusion. These include EBV, HHVs, HAV, and WNV. The SEN virus and vCJD prion may also be among them. EBV, HHV-6, HHV-7, and HHV-8 are all double stranded DNA viruses of the family *Herpesviridae*. EBV is the causative agent of infectious mononucleosis and Burkitt's lymphoma, and has been implicated in post-transfusion hepatitis.[23] A case in France of transfusion-transmitted infectious mononucleosis due to EBV was recently documented.[24] Although EBV is primarily spread through the saliva and most individuals have been infected by adulthood, transfusion-transmission has been problematic in a few cases, with the development in the recipient of subsequent infectious mononucleosis or post-transfusion hepatitis linked to a donor positive for EBV. In immunocompetent individu-

als, transfusion-transmitted EBV is asymptomatic; however, immunocompromised individuals who are seronegative may be at-risk for disease. Since EBV infects the B lymphocytes, leukoreduction may afford some protection to those at-risk. HHV-6 and HHV-7 cause exanthum subitum (roseola), a condition marked by high fever and convulsions in children. HHV-6 has also been associated with hepatitis and mononucleosis in adults.[25] Luppi reports that HHV-6 is prevalent in the leukocytes of blood donors, as does Kozireva.[26,27] Transmission of disease caused by HHV-6 through transfusion has yet to be established, however. Seropositivity in adults is very high and no current recommendations exist for protection of seronegative transfusion recipients.[28] HHV-7 DNA was found in the plasma of 10.6% of Latvia blood donors, although there are no current indications of transfusion-transmitted disease.[27]

HHV-8 is associated with Kaposi's sarcoma, Castelman's diseases, and other B-cell tumors.[29] HHV-8 transmission through organ transplantation has been demonstrated, although the primary route of transmission appears to be sexual.[30] One study in Tanzania showed that HHV-8 DNA can be detected in serum of the blood donor population.[29] Studies in both France and the U.S. showed that anti-HHV-8 is detectable in the serum of blood donors, with a seroprevalence in Texas blood donors of 15%.[31-33] A study of 100 donors in the Houston, Texas blood donor population failed to find HHV-8 DNA in any sample, although 25% of the donors had antibody to HHV-8.[31] Operskalski showed that in a group of 14 recipients of documented HHV-8 positive blood, none were seropositive 19 months after the transfusion.[34]

HAV is a single-stranded RNA virus belonging to the family *Picornaviridae*. It has traditionally been considered to be transmitted solely by the fecal-oral route and not by transfusion. However, in 1994, Vermylen and Peerlinck reported hepatitis A in hemophiliacs that was shown to have been transmitted through contaminated factor VIII concentrates.[35] As a result of this and other similar reports, U.S. plasma fractionators implemented NAT testing for HAV during 2001.[36] Although blood-borne transmission of HAV is apparently rare, with an incidence of approximately 1 in 1,000,000, transfusion-transmitted hepatitis A does occur.[37] Two cases of transfusion-transmitted hepatitis A from a single whole-blood donation that was prestorage leukoreduced were reported by Diwan in 2003.[38] The donor was diagnosed with Hepatitis A 18 days after making the donation. There is evidence that HAV viremia is present for up to 30 days prior to the onset of symptoms.[39] Therefore, calls have been made for the institution of HAV DNA NAT testing of blood donors in the U.S.[38] Implementation of such NAT testing is likely in the near future.[14]

WNV was first recognized in North America in 1999. It is a single-stranded RNA virus member of the Flaviviridae. The major vectors are mosquitos and the primary hosts are birds, although humans and other mammals can be infected when bitten by infected mosquitoes. The virus is fatal in 1 of 1,000 cases in humans. Several cases each of possible transmission by transfusion and transplantation have been reported. Three cases of donor to recipient transmission are well documented by the Centers for Disease Control (CDC). The first case was a multiorgan donor that received more than 60 units of blood products during treatment prior to her death. Four separate recipients of her organs all developed WNV disease through transplantation; none of the recipients had other possible exposures to WNV.[40] In a second case, a red cell recipient developed WNV and the virus was detected in the plasma from the same donation. The third case involved a donor from whom two recipients contracted WNV. A postpartum recipient of red blood cells from the donor with the disease developed WVN. A platelet concentrate made from the same donation as the red cells was later found to be positive for WNV. Based on these three separate, clearly documented cases, as well as several others, the CDC has called for the development of screening assays, for WNV.[41-43] The Canadian Blood Services plans to implement WNV testing of donors by summer 2003.[44] A major effort was made to develop and implement NAT testing for WNV RNA on donor units in the U.S. by the summer of 2003.[43]

Parvovirus B19, a single stranded DNA virus of the Parvoviridae family, is probably best known as the cause of fifth disease, also known as erythema infectosium, in children. Infection may also cause fulminant hepatitis even in immunocompetent individuals.[45] This virus is also associated with fetal infection and stillbirth when pregnant women are infected.[46] Infection of immunocompromised individuals may result in red cell aplasia.[47] Hayakawa and colleagues reported a case in which parvovirus B19 was transmitted by intravenous immune globulin.[48] Three cases of transfusion-transmitted B19 were documented by Azzi and others in 1999.[49] A group of French investigators described the presence of antibodies to parvovirus B19 in hemophiliacs.[50] With the detection of parvovirus B19 DNA in plasma pools and derivatives, the implementation of NAT testing of U.S. blood donors seems likely in the near future.[51]

Creutzfeld-Jacob disease (CJD) has been known for many years as a possible transfusion-transmitted degenerative disease. The classical form of CJD is familial 10% to 15 % of the time. The other 85% to 90% of cases are sporadic. The infection is thought to be caused by a prion, which is a protein particle. Classic CJD is very slowly progressive, with symptoms developing many years after infection. Once the symptoms develop, the disease then progresses rapidly from dementia and lack of motor skills to death. The incidence of CJD is extremely low, but donors have been asked about family history of CJD due to the demonstration of the prion in human leukocytes and documented transmission of CJD through injection of human growth hormone. The donor history questionnaire asks the donor to reveal any injection of human growth hormone; donors who have received it are indefinitely deferred. The recent development of a new variant form of this disease has sparked increased concern.

vCJD is a fatal degenerative disease first reported in England in 1996. The vCJD form is fatal very rapidly from the onset of the infection and has a much higher incidence than CJD. Approximately 140 cases had been reported world-wide by the end of 2002.[52] vCJD appears to be caused by the same infectious agent as bovine spongiform encephalopathy (BSE).[14,53] A study in the UK showed that it is possible to transmit BSE to a sheep by transfusion with whole blood taken from another sheep. The donor sheep was in the preclinical phase of an experimentally induced BSE infection and appeared healthy when the donation was taken.[53] Several other animal models have been used to show that transmission of related spongiform encephalopathies can be transmitted by transfusion; however, no human cases of transfusion-transmitted vCJD have been documented.[54] No testing is yet available to detect the causative agent, possibly a prion, in the blood of asymptomatic individuals. As previously noted, significant deferral mechanisms, including deferral of any donor who has traveled extensively or lived in continental Europe, are in place to try and prevent transmission.

APPARENTLY AVIRULENT TRANSFUSION-TRANSMITTED VIRUSES

SEN-V has been reported as a single-stranded, circular DNA virus. Standard sequencing studies have shown the existence of eight different genotypes of SEN-V, designated SEN-V A through H.[55] SEN-V is demonstrable through NAT testing; however, no test for SEN-V antibody is currently available.[56] A study in cooperation with the National Institutes of Health (NIH) showed that 92% (11 of 12) of patients suffering from transfusion-associated non-A to E hepatitis were infected with SEN-V after transfusion, compared with 24% (55 of 225) of recipients followed identically who did not develop hepatitis. Also shown in the same study was that the incidence of SEN-V infection after transfusion was 30% (86 of 286) while non-transfused controls had an incidence of 3% (3 of 97) and the prevalence of SEN-V in 436 volunteer donors was 1.8%.[57] Since the majority of infected transfusion recipients did not develop hepatitis, no real cause and effect of SEN-V in this regard has been proven. In a study by Pirovano, a similar incidence of SEN-V infection between HIV and HCV patients was shown at 45% and 46% respectively. It was found in this study that the prevalence of SEN-V infection was significantly higher (p <0.0001) among IV drug user than non-IV drug user HIV-positive individuals.[58] A study of several SEN-V positive transfusion recipients by Ball demonstrated sequence homology between donor and recipient, showing transfusion-transmission.[56] Since parenteral transmission is apparent, SEN-V has been studied further for implication in post-transfusion hepatitis. SEN-V viremic individuals on hemodialysis were studied by two different groups, one in Germany and one in Japan. Each showed that, while SEN-V infection is common in hemodialysis patients, none of those studied by either group of researchers developed hepatitis without co-infection with HCV. Also, both groups showed that SEN-V infection was not associated with the amount of transfusion.[59,60] Currently, it appears that, while SEN-V is transfusion-transmitted, there is no clear association with production of disease.

HGV, also known as GBV-C, is an RNA virus of the Flaviviridae family. HGV has a 29% amino acid homology with another Flavovirus, HCV. Several studies have documented that transfusion-transmission and coinfection with HCV is common.[61-64] The virus has been found in from 1% to 2% of the donor population in the U.S.[64] In Germany, both high levels of antibody to HGV (17.5%) and high levels of viremia (19.6%) in hemodialysis patients were seen. These patients had a significant (p <0.05) increase in incidence of HGV infection when more than five transfusions had been received.[65] High frequencies of HGV were also reported in Japanese blood donors, with 15.8% of 203 donors tested being positive for HGV RNA.[66] The site of HGV replication does not appear to be in hepatic cells, since HGV is not hepatotropic, but may occur in the mononuclear cells of the spleen or bone marrow.[67] There is a documented absence of correlation between infection with HGV and clinically significant liver disease.[68-70] A study in Italy showed that, while HGV infection is common among kidney transplant patients, chronic liver disease was not increased in these pa-

tients as compared to kidney transplant patients that were HGV negative.[71] Studies of patients with acute non-A through E hepatitis in Sicily demonstrated that only 35% (19 of 54) were infected with HGV and none of the HGV infected patients progressed to chronic liver disease.[72] No association between HGV positivity and ALT or gamma glutamyl transferase (GGT) elevations were seen in a study of peritoneal dialysis patients in Italy.[73] In one study, HGV or GBV-C RNA was the only viral marker seen in three patients with a mild form of hepatitis. These three patients, out of 79 studied, had only slightly elevated ALT levels.[64] However, a pediatric patient in Greece who developed fulminant hepatic failure was shown to be positive for HGV RNA and negative for all other known hepatitis viruses.[74] Also, a study in China showed that 52 patients infected with HGV had no other hepatitis viral markers present, yet showed pathological liver changes.[75] Thus, these results indicate that the pathogenicity of HGV is unclear at present and more research is necessary to determine whether or not this virus is a causative agent of post-transfusion hepatitis. There appears to be no evidence that HGV causes any other clinical disease state.

TTV is a single-stranded DNA virus of the Circoviridae family. It was initially reported in patients with posttransfusion hepatitis of unknown etiology. The virus appears to be prevalent in the healthy population, with a rate of 65% (36 of 55) in healthy, previously non-transfused children studied in the Washington DC area.[11] TTV seems to be transmitted primarily by the oral-fecal route in children under five years of age. The virus is transfusion-transmissible; however, this route is not a major source of the virus epidemiologically.[76] No specific pathogenicity of this virus has been described although an association with posttransfusion hepatitis has been explored.[77-80] In a Chinese study of the prevalence of TTV in blood donors, 36 out of 340 (10.6%) blood donors tested were positive for TTV. Prior to transfusion 11 of 130 (8.5%) recipients of these units were positive for TTV. Of the 119 recipients of the blood that were TTV negative prior to transfusion, 18 were positive after transfusion; however, only one of the corresponding donors was reported to be TTV positive.[81] The prevalence of TTV DNA in blood donors screened using a specific type of TTV primers is as high as 80%.[77] TTV, therefore, appears to be prevalent in the healthy population without causing disease.

ANOTHER POSSIBILITY?

HPV may be transfusion-transmissible. An interesting report from Brazil concerns the transfusion transmission of BPV in cattle. BPV is a virus with similarities to HPV. The documentation of the transmission of BPV both through blood transfusion and vertically may be of significance.[82] Although only one paper regarding such transmission can be found, it raises interesting possibilities. The vertical transmission of HPV has been suggested by Sedlacek and colleagues.[83] HPV DNA has been demonstrated in the lymphocytes of women with cervical cancer.[84] Whether or not this is an indication that we may find yet another transfusion-transmissible virus remains to be seen.

PREVENTION BY VIRAL INACTIVATION

Inactivation of pathogens in plasma products has been in use for several years. The solvent-detergent (S-D) process was initially licensed in 1985 for use in the manufacture of factor VIII concentrates. To use this process on fresh frozen plasma (SD-FFP), pools of between 380 and 2500 ABO identical units are made after thawing and the plasma is filtered to remove cell debris. Treatment with a 1% tri (*n*-butyl) phosphate (TNBP) solution as solvent, containing Triton X, Tween 80, or sodium cholate detergent is commonly used. The treatment is performed at 30 °C for four hours. The detergent disrupts viral lipid envelopes. After treatment the solvent must be removed. The plasma is then sterile filtered and frozen in 200 mL aliquots. HCV, HBV, HTLV-I and II, HIV, HGV, and WNV are all enveloped viruses and most have been shown to be reduced considerably by S-D treatment. Many transfusion-transmitted viruses, such as HAV, HPV, and parvovirus B19, are either non-enveloped or their envelopes have not been detected, and have not been shown to be removed by S-D treatment.[85-87]

Methylene blue treatment of FFP to inactivate viruses was first described in 1991. This phenothiazine dye has been shown to inactivate retroviruses, herpes viruses, HCV, HBV, and WNV in both plasma and red cells.[88] Leukocytes are first removed from the plasma, either by filtration or freezing and thawing. After leukocyte removal and the addition of methylene blue to a concentration of 1μM, the units of plasma are exposed to white light. This photoinactivation process is performed on single units, as opposed to large pools used for S-D FFP preparation.[87] Methylene blue and other photodynamic inactivators have also been studied for use in platelets, but appear to damage the platelets in the process.[85,89,90] In red cell concentrates, photodynamic processes tested thus far cause red cell damage. A phenothiazine compound, dimethymethylene blue (DMMB) is comparable or better than methylene blue for inactivation of viruses but does not cause as much red cell damage.[91]

Psoralens may also be used to inactivate viruses in FFP and in platelet concentrates. The psoralens, such as aminomethyl trimethyl psoralen (AMT) and S-59, added to the plasma will intercalate with nucleic acid and, when then exposed to ultraviolet A (UVA) light for three or four minutes, will form a stable photoadduct between nucleic acids strands and pyrimidine bases. After the procedure is completed, the residual S-59 and unbound products must be removed. This process can be used for single or jumbo units of plasma for FFP. HIV, HCV, and several other viruses have been shown to be inactivated by this process. S-59 may have an advantage in that prefiltration and freeze-thaw steps to remove leukocytes are not needed and bacterial inactivation has also been shown to occur.[85,87]

Pastuerization has also been used successfully to inactivate viruses in plasma products such as IVIG and coagulation factors, but not in FFP. All lipid-enveloped viruses are inactivated as well as several non-enveloped viruses. Time and temperature, as well as protein concentrations must be monitored when this method is used.[86]

Caprylates, naturally occurring fatty acids found in animal and vegetable fats, have also been studied for virus inactivation in IVIG and albumin plasma products. Caprylate is added to facilitate the precipitation of unneeded proteins at 2 °C to 8 °C, then, by readjusting the concentration and raising the temperature to 25 °C, enveloped viruses were inactivated. When HIV-1, and virus models for HCV and herpes viruses were tested, caprylate inactivated them in IVIG products as well as S-D methods did.[92]

Frangible anchor length effectors (FRALE) have also been developed.[93] FRALEs are molecules that are activated by a shift in pH after they are added to packed red blood cells and then form covalent adducts with DNA and RNA. The compound tested by Cook and colleagues inactivated HIV, several other viruses and both gram-negative and gram-positive bacteria. Posttransfusion red cell recovery using FRALE-treated RBCs in both murine and canine models was comparable to posttransfusion recovery of untreated RBCs. Clinical trials are underway.[85,93]

Other processes to remove infectious agents from immune globulin preparations include nanofiltration, which has been shown to remove prion material, antibody-coated parvoviruses, and antibody-coated enteroviruses. This process may show some promise in eliminating the non-enveloped viruses and prions from transfused products.[94,95]

Transfusion-transmitted viruses, while increasingly being discovered and recognized, are also being tamed. New viruses emerge routinely, however, and require vigilance from the transfusion community. As demonstrated by the furor surrounding the advent of transfusion-transmitted HIV, the public demands safety of the blood supply approaching zero percent viral transmission. The blood banking community has accepted this challenge. New testing is constantly being implemented to insure an uninfected blood supply. New methods for treating blood components to inactivate viruses show promise in the fight against transmission, and FFP is routinely treated prior to distribution. Inactivation of viruses in platelet concentrates and packed red cells is on the horizon.

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