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Emerging Infectious Diseases That Threaten the Blood Supply

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Following the devastating effects of blood-transmitted human immunodeficiency virus (HIV), blood establishments have become increasingly vigilant for the emergence or re-emergence of new threats to the safety of the blood supply. Many agents have fulfilled the broad definition of emerging blood-transmitted infections, including West Nile virus (WNV), *Trypanosoma cruzi, Plasmodium* spp., *Babesia* spp., parvovirus B19, dengue virus, and the prions that cause variant Creutzfeld-Jacob disease (vCJD). Other agents such as human herpes virus- 8 (HHV-8—Kaposi's sarcoma virus) and Borellia (Lyme disease) and, perhaps, avian flu virus, are known to have a viremic phase, but have not yet been proved to be transfusion-transmitted. In the wake of these threats, transfusion services use a variety of donor screening interventions, including serologic assays, nucleic acid assays, and geographic exclusions based on potential exposure. The ultimate safeguard may be a pre-emptive pathogen inactivation strategy that will disrupt all nucleic acid–containing agents (though not prions). Considerable effort and resources have been invested in this arena, but currently no single technique is effective for inactivation of both liquid and cellular blood products and toxicity issues have not been completely resolved. The blood supply is remarkably safe with the risk of major pathogens such as hepatitis C virus (HCV) and HIV now reduced to less than one transmission per 2 to 3 million exposures. However, to approach near-zero infectious disease risk for emerging and re-emerging pathogens, new strategies such as pathogen inactivation or multi-pathogen microarray technology will need to be developed or refined.
Semin Hematol 44:32-41 © 2007 Elsevier Inc. All rights reserved.

Emerging infections have several mechanisms of evolution. First, previously unrecognized human agents can appear de novo in a population, often by adaptations from animal to human hosts. Such zoonotic transmissions have caused devastating diseases, best exemplified by human immunodeficiency virus (HIV) that evolved from simian viruses in Africa and variant Creuztfeld-Jacob disease (vCJD) that evolved sequentially from prion diseases in sheep (scrapie) and cows (bovine spongiform encephalopathy). Second, long-recognized agents have emerged as new disease threats due to changing population dynamics or altered migration patterns of intermediate hosts and vectors; examples are West Nile virus (WNV) infection in the United States due to migrations of susceptible bird hosts and *Trypanosoma cruzi* infections (Chagas disease) that has followed human migration from Central and South America into the United States. These agents are more re-emerging than emerging, but they create emerging problems. Third, established agents can present emerging problems because of changes in the environment that foster growth of the agent or its vector. In the transfusion setting, sepsis now occurs related to the room temperature storage of platelets that facilitates bacterial growth. Fourth, existing agents that have caused little or no human disease may emerge as threats when mutational adaptations facilitate animal to human and then human to human spread. The major current concern in this category is avian flu, strain H5N1, with its potential to create a pandemic.

The Institute of Medicine has defined emerging infections as, "new, re-emerging or drug-resistant infections whose incidence in humans has increased within the past two decades or whose incidence threatens to increase in the near future."

Multiple agents fall under this definition and are known to be, or have the potential to be, transmitted by blood transfusion. These agents traverse all taxonomic classifications of

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doi:10.1053/j.seminhematol.2006.09.016

Table 1 Key Emerging Infections That Are, or May Be, Transmissible by Blood Transfusion

Organism (disease)	Normal Transmission Route	Transmissibility by Transfusion/Transfusion Risk	Transfusion Cases in US	Treatment for Disease	Intervention for Transfusion
Parasites					
Babesia	Ticks	Known/moderate	>50	Yes	NA
Leishmania	Sandflies	Anectdotal	0	Partial	Q
Malaria	Mosquitoes	Known/low	1–2 per year	Yes	Q, T (unlicensed)
T cruzi (Chagas)	Reduvid bugs	Known/low	5	Partial	T (unlicensed)
Bacteria					
Anaplasma (ehrlichiosis)	Ticks	Probable/infrequent	1 (presumed)	Yes	NA
Gram$^+$ & gram$^-$ species	Multiple	Known/High	1:75,000 apheresis platelets	Partial	T
Spirochetes					
Borrelia burgdorferi (Lyme disease)	Ticks	Theoretical	0	Yes	NA
Viruses					
Avian influenza	Respiratory, droplet	Theoretical	0	Partial	NA
Dengue (dengue fever)	Mosquitoes	Known/low	0 (1 in Hong Kong)	No	NA
HHV-8 (Kaposi's sarcoma)	Saliva, sexual contact	Probable/low	0	No	NA, (LR?)
Parvovirus B19 [erythrovirus] (aplastic crises, RBC aplasia)	Respiratory	Known/low	3–6	Yes	(T)
SARS-coronavirus	Respiratory, fecal-oral	Theoretical	0	No	Q
Simian foamy virus (No known disease)	Primate contact	Theoretical	0	No	Q, (LR)
TTV/SEN-V [circoviruses] (No known disease)	Parenteral	Known/high	Very frequent (up to 25% of recipients)	None required	NA
GBV-C/HGV [flaviviruses] (No known disease)	Parenteral	Known/high	Very frequent (up to 7% of recipients)	None required	NA
New variants of established agents (e.g. HBV, HIV)	Parenteral	Known/low	Rare	Partial	Q, (T)
Prions					
vCJD	Oral	Known	0 (3 in England)	No	(F)
Chronic wasting disease	? oral	Unknown	0	No	NA

Abbreviations: NA, not available; Q, donor questioning; T, laboratory test; (T), test may be partially effective; (LR), leukoreduction thought to reduce infectivity, (F), affinity filters under development.

infectious agents and cause a diverse constellation of illnesses. Table 1 summarizes the major proven or suspected emerging transfusion-transmitted agents. From this panoply of agents, we have chosen to focus on those that exemplify the different patterns of emergence described above and, specifically, have selected parasitic infections due to malaria, *Babesia* spp., and *Trypanosoma cruzi*, viral infections due to WNV, dengue fever virus, erythrovirus (parvovirus B19), human herpes virus-8 (HHV-8), and avian flu virus and the prion disease, vCJD. We will also discuss pathogen reduction strategies for transfused products and the concept of a zero-risk blood supply.

Parasitic Infections

Malaria

Malaria is caused by the intraerythrocytic protozoan parasites *Plasmodium vivax*, *P falciparum, P ovale, and P malariae*, each of which can cause clinical illness as well as asymptomatic infection. *P falciparum* can cause severe disease, manifest not only by episodic fever and diaphoreses but also by severe anemia, central nervous system disturbance, renal failure with massive hemoglobinuria ("black water fever"), and fatal shock syndrome. Malarial infections result in an enormous disease burden, with an annual incidence of 300 to 500 mil-

lion cases worldwide; it is a major cause of infant death in endemic regions. The anopheles mosquito is the vector for most transmissions, but in the United States, the receipt of blood from an infected but asymptomatic donor from a malaria-endemic area is a more common route than is a direct mosquito bite.

An ancient infection, malaria is included as an emerging transfusion-transmitted infection because it has spread from endemic to non-endemic regions, the result of escalating emigration from previously remote countries facilitated by the increasing availability of air travel and recurrent international conflicts that transport soldiers to and from endemic areas. Malarial spread by transfusion is also fostered by the relatively long asymptomatic parasitemia for each of the four major malarial species. However, despite this constellation of circumstances, relatively few transfusion-transmitted cases occur. The Centers for Disease Control (CDC) reports 103 transfusion-associated cases in the United States in the four decades from 1958 to 1998, averaging two to three cases per year or one case per 4 million units transfused.[1] These numbers may underestimate true infections because some remain undetected and others remain unreported. Transfusion-transmitted malaria remains a risk because latent infection with *P falciparum* can persist on average for 1 to 2 years and considerably longer periods have been described.[1] Infection with *P vivax* and *P ovale* can persist as long as 5 to 7 years, and *P malariae* for a lifetime. Malaria is also a blood-transmitted risk because the organism can survive at least a week at 4°C and can survive frozen red cell storage.

No blood donor screening assay for malaria is currently licensed in the United States. Both antibody and nucleic acid detection strategies are feasible and under development, but they would have a very high cost-benefit ratio, and the former might have low specificity and sensitivity. Presently, blood banks depend on donor screening questions to exclude persons at risk because of lifelong or temporary residence in endemic areas. Donors are deferred on the following bases: (1) permanent residents of non-endemic countries who travel to an endemic area (as defined by CDC) are deferred for 1 year from the time that they leave the endemic area whether or not they have received anti-malarial prophylaxis, provided that they have been free of symptoms suggestive of malaria; (2) immigrants, refugees, and citizens or other residents of endemic countries are deferred for 3 years from the time they departed the endemic area provided that they have been free of symptoms to suggest malarial infection; (3) persons with a history of malaria are deferred for 3 years after symptoms are no longer present. These deferral periods are based on CDC data indicating that 97% of malaria cases in travelers occur within 1 year of leaving the risk area and that 99% of cases in permanent residents of endemic areas occur within 3 years of leaving the endemic area.[1] While these deferrals have been effective in limiting transfusion-transmitted malaria, they come at the cost of many unnecessary donor deferrals in an age where travel to endemic areas is quite common. A specific and sensitive assay to detect parasitemia would be sparing of donors, though at a high cost relative to benefit.

Babesia (babesiosis)

There are a number of different species of *Babesia* worldwide. They are protozoan parasites of the class *Piroplasmea* (or *Sporozoea*), order *Piroplasmida*, or more loosely, piroplasms. The agents resemble malaria parasites and babesiosis may be mistakenly diagnosed as malaria. In the United States, *B microti* is the most commonly identified agent, although *B divergens* is also present. At least two other unnamed species have been identified in Washington and California and are respectively known as WA-1 and CA-1.[2]

Babesia spp. infect numerous mammalian species and are transmitted by ticks, primarily the *Ixodes* genus. *B microti* is transmitted by *Ixodes dammini* (also known as *I scapularis*), the black-legged or deer tick. The primary mammalian reservoir is the white-footed mouse, but the tick may also be transported by deer. *B microti* has a restricted, but expanding geographic range in the United States; it is largely confined to Northeastern coastal areas and parts of the upper Midwest, including Minnesota and Wisconsin. *B divergens* also has been identified in some US states, but the tick vector has not been identified. The human is always an accidental host, acquiring infection through a tick-bite, which frequently goes unnoticed, as *Ixodes* ticks are very small. Ticks have to attach to their host for 1 to 3 days in order to effectively transmit the parasite.

Despite the global distribution of *Babesia* spp., almost all reported cases of transmission by transfusion have occurred in the United States, with more than 50 documented occurrences. Data on the prevalence of *Babesia* infection in US blood donors from areas of high endemicity are reasonably sound. In the northeast United States, the seroprevalence of *B microti* reportedly ranges from 0.3% in the general population of Connecticut to 9.5% in patients with Lyme disease. Several reports of *B microti* in blood donors have been published, with rates as high as 4.3% (five of 115 positive) on Shelter Island, NY. A recent Connecticut study reported that 30 of 3,490 (0.9%) blood donors were seropositive for *B microti*. Perhaps more important, 10 of 19 (53%) seropositive donors from this study were demonstrably parasitemic when tested by polymerase chain reaction (PCR), indicating an obvious transmission risk.[2] A relatively recent finding is that parasitemia may be quite prolonged (as much as 6 months or more), even among asymptomatic individuals. The estimated risk of transfusion-transmission is as high as one in every 1,800 transfused units in highly endemic areas of Connecticut.[2] Interestingly, some transmission has occurred from platelet components, which presumably contained contaminating red cells.

The incubation period after a tick bite is usually 1 to 6 weeks but occasionally as long as 3 months. Manifestations range from asymptomatic infection to a fulminant, malaria-like infection that rarely may be fatal. Symptoms are generally flu-like and may include malaise, chills, myalgia, anemia, fatigue, and fever (which can approach 40°C). Some patients also have nausea, emesis, night sweats, weight loss, and hematuria, which are believed to be associated with high levels of parasitemia. Hepatomegaly and splenomegaly may also be

present. The parasites primarily infect erythrocytes. In most cases, infection and disease are acute, but as pointed out above, there may be some degree of chronicity. Immunocompromised, aged, and asplenic patients are at particular risk of developing serious, even fatal, disease as a result of transfusion transmission of the agent. While usually self-limiting, more severe cases are treatable with clindamycin and quinine; more recently, a combination of atovaquone and clindamycin has been shown to be effective, with fewer side effects.[3]

Disease manifestations and potential exposure history contribute to an initial diagnosis, but exposure may be inapparent. A history of blood transfusion (particularly in an endemic area, although cases have occurred as a result of blood imported from endemic areas) may raise suspicion. Laboratory diagnosis should include examination of stained blood smears and tests for antibodies using immunofluorescence. PCR also may be helpful, but these procedures are available only on an investigational basis. Donor screening tests are not currently available.[2]

At present, no measures effectively prevent transfusion transmission of *Babesia*. While a history of tick-bite would appear to be a useful screen, it is neither sufficiently sensitive nor specific.[4] Travel history suffers from the same problems, and the possibility of prolonged parasitemia reduces the efficacy of restricting donations from areas of high endemicity during the tick season. Clinicians in areas of high risk should be aware of the possibility of transfusion-associated *Babesia* infection and its treatment.

Trypanosoma *cruzi* (Chagas disease)

Chagas' disease is caused by the protozoan parasite, *Trypanosoma cruzi*. The parasite infects many mammalian species, including man. *T cruzi* is similar to other trypanosomes in its passage through a number of different morphologic forms, depending on the stage in its life cycle. The parasite is transmitted by reduvid or triatomine bugs. The parasite multiplies to high levels in the gut of the bug and bug feces, shed during feeding, are heavily contaminated. Infection (at least in humans) usually results from rubbing the insect's feces into the site of the bite or into mucus membranes. Congenital infection may also occur from an infected mother. Recently, oral transmission of *T cruzi* has been documented. In areas that are not endemic for *T cruzi*, blood transfusion may be the predominant route of human infection. A number of cases of transmission by organ transplantation have also been reported.[5]

The parasite occurs in the continental Americas between latitude 40S and 40N. However, conditions favoring natural transmission to humans are generally confined to parts of Latin America, because of the high prevalence of infection among feral and domestic animals and the vector insect's tendency to live in cracks and fissures and roofs of substandard housing. Seroprevalence rates vary widely but may be as high as 60% in parts of Bolivia.[6] An estimated 19 million infected individuals live in South and Central America and parts of Mexico. In many South American countries, significant efforts to reduce the risk of disease, usually by aggressive use of insecticides inside homes, are underway. In the United States, although rare autochthonous infections have occurred, the majority of human infections follow from immigration of individuals infected elsewhere. At one time, an estimated 100,000 legal immigrants were infected; that number may be much higher now. Seroprevalence studies have indicated that about 1 in 7,500 to 1 in 9,000 blood donors may be seropositive in areas with large numbers of migrants, such as Los Angeles or Miami,[7] but the overall US prevalence may be lower, 1:25,000 to 1:50,000. In many cases, infection is lifelong; about 60% of seropositive donors in the United States have parasitemia as demonstrated by PCR and/or culture, despite first infection many years earlier.

At least seven cases of transfusion transmission of *T cruzi* have been documented in the United States and Canada, as have three or four clusters of infection via organ transplantation. Immunocompromised patients seem to be more at risk of serious or fulminant disease. Efforts to identify transfusion transmission by lookback or by evaluation of transfused patients have not revealed additional cases, so the risk of infection may not be as high as is suggested by seroprevalence rates.[7]

Acute disease is generally mild and treatable using the experimental drugs nifurtimox and benznizadole; however, both drugs have relatively serious side-effects. In the United States, they are available through the CDC. Infection often takes place early in life. Most individuals go on to chronic, lifelong infection, which cannot be treated. Although chronic infection is often asymptomatic, in 20% to 30% of cases, it leads to serious or fatal cardiac or intestinal disease. Cardiac infection may lead to arrhythmias and sudden cardiac death; less frequently, infection of the smooth muscle of the intestine can cause megaesophagus or megacolon.

Parasites may be visualized in the blood or in infected tissue. Tests for antibodies to *T cruzi* are available commercially or from reference laboratories. Indirect immunofluorescence or EIA formats are most common, and a passive hemagglutination assay has been developed and commercialized. A variety of confirmatory tests are available, but none appears to be a gold standard. In the United States, enzyme immunoassay tests for blood screening are under development and one or more will likely be licensed and available in 2007–2008.

All US blood donors are asked if they have had Chagas' disease, a question of little, if any, value. A history of birth or prolonged travel in endemic areas is not adequately specific and would decimate the blood supply in some locations. Chagas blood screening assays will likely be employed once they are licensed. Limited data suggest that leukoreduction may have some effect on parasite titers in blood, but even this approach would not offer complete protection.

Viruses

West Nile Virus

WNV is an enveloped, single-stranded RNA flavivirus in the Japanese encephalitis group. WNV primarily infects numer-

ous species of birds, which act as the amplifying host. However, many other mammals and some other vertebrates can be infected. The virus is transmitted by mosquitoes (largely culicines) and man is an accidental host. Until 1999, the virus was confined to Africa, Southern Europe, and parts of the Middle East and India, but it appeared in the United States for the first time that year, when 62 human cases were reported in Queens, NY. Over the next 5 to 6 years, the virus spread throughout the United States and into Canada, Mexico, and some of the Caribbean. To date, peak years of the epidemic have been 2002 and 2003, with 4,156 and 9,862 reported human cases, respectively, but there are estimates that several hundred thousand individuals were infected in each of those years. Fewer cases were seen in the 2 subsequent years. The risk of transfusion transmission was estimated as high as one per thousand units in areas and times of peak incidence.[8] Some 23 documented cases of transmission by transfusion occurred in 2002[9] and, as a result, nucleic acid testing (NAT) for WNV RNA was implemented by July 2003. This testing has identified more than 2,000 potentially infectious components in the first three years of use.[10]

WNV infection is asymptomatic in approximately 80% of cases; West Nile fever occurs among most of the remainder, sometimes associated with headache, eye pain, fatigue, muscle aches, and rash. Symptoms may last a few days to several weeks. More severe disease is usually neuroinvasive and may manifest with severe headache, high fever, neck stiffness, stupor, disorientation, coma, tremors, convulsions, muscle weakness, and paralysis. This meningoencephalitis may be fatal and is more common in persons over age 50 and in immunocompromised individuals. An estimated one in 150 persons infected with the WNV will develop severe disease that can be fatal or lead to prolonged disability.

In asymptomatic or mild disease, viremia has been reported to persist 2 or more months. However, relatively high levels of viremia in the absence of antibody rarely last more than 2 weeks, and this is the period during which an individual might be infectious via blood transfusion. Tests for IgM antibodies to WNV are used to establish a laboratory diagnosis in the presence of symptoms. Additionally, the virus may be isolated in Vero cells, but PCR or other NAT for viral RNA is more sensitive. NAT is used for blood donor screening in the US. Donors are deferred for 120 days after WNV disease or a positive test.

Dengue Virus

Dengue virus is an enveloped, single-stranded RNA virus belonging to the flavivirus group. Particles are 35 to 42 nm in diameter. There are four serotypes, identified as 1–4. The virus is transmitted by mosquitoes (predominantly *Aedes aegypti* and *A albopictus*) and is endemic to the tropics. Humans are the primary amplifying host, although monkeys may also be infected. Recently, large outbreaks of dengue have occurred in a number of countries, particularly in Southeast Asia.

Dengue infection produces a number of symptoms, many of which are similar to those of West Nile fever, including fever, headache, eye pain, and rash, but in addition there is often severe joint and muscle pain. Infection with one strain of the virus results in an effective immune response to that strain, but second infection with a differing strain can still occur. Second infections can result in dengue hemorrhagic fever, a more serious disease with more pronounced symptoms accompanied by hemorrhage. A shock syndrome can ensue and the mortality rate is high.

There are no well-documented cases of transfusion-transmission of dengue; one case was reported from Hong Kong but not in the scientific literature (Pro-Med-mail, October 11, 2002). It is possible that the asymptomatic viremic phase of dengue is much shorter than that for WNV; a transfusion-transmitted case likely would not be recognized in the context of a large, mosquito-borne outbreak. There is a possible instance of dengue transmission to a bone marrow transplant recipient in Puerto Rico, but it is unclear if this case truly represents transfusion-transmission. Recently, studies have shown detectable viremia in blood donations from Brazil and Honduras (Busch MP, personal communication, 2006).

There are no obvious interventions to manage the potential for transfusion-transmitted dengue, but NAT screening tests could be developed and implemented if such intervention were necessary. Such a strategy would be preferable to the prohibition of blood collection, as implemented in Northern Queensland, Australia as a result of the introduction of dengue into that area.

Parvovirus B19

Parvovirus B19 merits discussion not because it is a newly emerging agent but because its potentially severe pathologic effects have become more apparent in the past decade with widespread use of pooled plasma components that foster its spread by transfusion.[11] Parvovirus B19 is not inactivated by the solvent detergent method that renders pooled plasma free of the major lipid-enveloped agents. The increasing number of immunocompromised blood recipients who are susceptible to prolonged B19 viremia and the serious bone marrow depressive effects of this agent make infection more widely appreciated.

Parvovirus B19, now designated erythrovirus, is a member of the *Parvoviridae* family of small (19 to 23 nm), nonenveloped, single-stranded DNA viruses. *Parvoviridae* infect many insect and animal species, but only B19 has been associated with significant human disease. The major cellular receptor for parvovirus B19 is globoside, a glycosphingolipid found predominantly on erythroid cells or their progenitors. Interestingly, the glycosphingolipid that constitutes the viral receptor is the long-recognized P antigen on red cells; rare individuals who lack P antigen on their red cells (p or Tja^- phenotype) are resistant to B19 infection.

Parvovirus B19 is responsible for a common, benign exanthema of children known as erythema infectiosum or fifth disease. More significantly, B19 infects erythropoietic progenitor cells and can cause a transient, but sometimes profound, aplastic crisis in patients with underlying hemolytic syndromes, particularly sickle cell anemia and hereditary

spherocytosis; indeed, an aplastic crisis occurring in a patient with a known hemolytic anemia is preponderantly due to parvovirus B19 and sometimes, a compensated hemolytic anemia is first recognized when there is a superimposed B19-induced transient aplastic crisis. In addition, parvovirus B19 can cause severe, prolonged red cell aplasia in patients with congenital or acquired immunodeficiency because such patients have persistent high-level viremia in the absence of antibody.[11] The number of red blood cell precursors lysed and the duration of the viremic destructive phase in immunocompetent individuals with no underlying hemolytic state are usually insufficient to cause detectable anemia, but temporarily decreased red cell production can be demonstrated.[11] Parvovirus B19 can cause fatal hydrops fetalis, especially when the fetus is infected during the second trimester. Parvovirus B19 infection is responsible for an estimated 10% to 15% of all cases of non-immune hydrops fetalis. In adults, especially women, an associated arthropathy can occur in 50%; arthropathy is usually transient but can persist or recur even in the absence of detectable viremia. Surprisingly, parvovirus B19 infection also can be associated with neutropenia, frank agranulocytosis, and thrombocytopenia. Whether these associated cytopenias indicate permissive receptors on non-erythroid progenitors or simply a bystander effect is unknown.

Parvovirus B19 transmission is most common in the spring, predominantly by the respiratory/droplet route; transmission is most likely in the high viremic phase that precedes clinical disease. Secondary transmission in households and schools is very efficient. Parvovirus B19 infection is unequivocally spread by blood transfusion, particularly pooled plasma components, but the number of transmissions compared to the number of infections is very small because most viremic individuals have low-titer virus that coexists with neutralizing antibody. IgM antibodies to B19 appear 10 to 14 days after infection and can persist for several months; neutralizing IgG antibodies generally appear about 2 weeks after infection and persist for life.[12] The protective role of antibodies to parvovirus B19 is demonstrated in the efficacy of commercial immunoglobulin used to treat B19 infection in immunocompromised subjects. Recipient infections stem primarily from donors who are early in infection and have high levels of viremia, either in the absence of antibody or far in excess of the neutralizing potential of the emerging antibody. In the absence of antibody, viral levels can be as high as 10^{14} copies/mL. Although the prevalence of parvovirus B19 viremia in donors has been reported to range from 0.03% to 0.1%, only three cases of anemia associated with blood transfusion were reported in the United States and Europe over a 5-year period.[13,14]

Although the frequency of clinically significant parvovirus infection is low, the severe clinical consequences in persons with hemolytic anemia or immunodeficiency warrant preventive measures where possible. Anti-parvovirus antibody is too prevalent (>50%) in the population to utilize as a donor screening assay and thus detection depends on nucleic acid amplification. Currently, there is no requirement to perform NAT of standard blood components and it is unlikely that such a recommendation will be forthcoming. However, since the greatest risk product is pooled plasma, commercial plasma fractionators have elected to perform parvovirus NAT testing using a "de-tuned" or insensitive assay that only will detect levels of virus greater than 10^4 copies/mL. As noted above, low levels of viremia exist primarily in the presence of coexistent neutralizing antibody and only high levels of virus transmit infection and cause disease in susceptible recipients. Perhaps the main "emerging" concern is that immunosuppressed individuals constitute an increasing proportion of patients who require blood products; even a relatively low frequency of transmission in this population could have devastating consequences. This virus also poses a particular threat to the fetus, raising special concern for in utero transfusion or maternal transfusion during early pregnancy.

Human Herpes Virus-8

HHV-8 is the etiologic agent of Kaposi's sarcoma, accounting for its other name, Kaposi's sarcoma-associated herpes virus (KSHV). HHV-8 is formally a gamma herpesvirus and is enveloped, with double-stranded DNA. Viral particles are 180 to 200 nm in diameter.

The virus is naturally transmitted though saliva and sexual exposure and also has been transmitted by organ transplantation. There is significant controversy over the accuracy of available serologic tests for HHV-8 infection, so the prevalence is uncertain. Using IFA, donor populations in the United States have an antibody prevalence of 2.5% to 3%, significantly lower than that for most other human herpesviruses.[15] Much higher rates are seen among patients with Kaposi's sarcoma and in HIV-positive male homosexuals. The prevalence is also higher in areas such as Africa and southeast Europe, where there is a high frequency of classical Kaposi's sarcoma. It is unclear whether any particular group of patients is at increased risk of clinical disease from HHV-8. The appearance of Kaposi's sarcoma in organ transplant recipients suggests that immunocompromised patients may be at increased risk. HHV-8 transmissibility by transfusion is not unequivocally established but seems likely. A number of studies have shown increased prevalence of infection in intravenous drug users and selected transfused populations along with some convincing evidence of transfusion-associated seroconversion.[16] Dollard and colleagues (http://www.fda.gov.cber/minutes/nat030806t.htm) provide the best evidence to date of transfusion-transmission in a large study conducted in Africa.[17]

HHV-8 is the etiologic agent of Kaposi's sarcoma and perhaps of other proliferative diseases, such as multicentric Castleman's disease. The pathologic mechanisms are poorly understood, but are presumably consistent with proliferative diseases caused by other herpes viruses. Infection is chronic and resultant disease, if it occurs, requires many years to develop. Most infections are likely to be asymptomatic. There is no information about acute manifestations of infection. Among asymptomatic, seropositive individuals, the detection of viral DNA by PCR is infrequent.

HHV-8 infection can be identified by several serologic assays for antibodies, supplemented by PCR for viral DNA.

There are no tests currently available for blood bank screening, nor is there agreement as to whether such screening is necessary or desirable. Blood from HHV-8–infected individuals probably has been transfused for many years without documented obvious adverse outcomes. HHV-8 is cell-associated, and any potential infectivity should be reduced or eliminated by leukoreduction.

Avian Flu Virus

Avian influenza virus, strain H5N1, is a single-stranded, enveloped, RNA virus in the family *Orthomyxoviridae* and the genus, influenza virus A. Influenza strains are classified by their surface hemagglutinin (H) sequence (H is essential to binding to host receptors) and by their neuraminidase (N) sequence. Avian influenza viruses do not typically infect humans because their hemagglutinin configuration cannot bind to the human receptor on respiratory epithelium. Avian flu due to the H5N1 strain has emerged as a massive epidemic among chickens and other fowl in eastern and Southeast Asia and, because of the migration patterns of birds, is now spreading globally. Millions of commercial fowl have been slaughtered to contain the epidemic. Transmission from birds to humans has been increasingly documented, almost always among persons who had direct contact with infected fowl. Human to human transmission has been very limited, but there have been some household clusters[18] and one case of apparent child-to-mother transmission.[19] Serologic studies among household contacts[20] and exposed healthcare workers do not support efficient human to human spread. Nonetheless, there is great concern that the agent will mutate by random combination to more easily spread among humans, raising the specter of a global influenza pandemic. Governments and public health services throughout the world are preparing for an eventuality that some believe is inevitable and others consider highly unlikely. The difference in opinion hinges on recombinatorial events that would foster respiratory spread of H5N1 similar to that seen with the annual waves of seasonal flu. H5N1 is anticipated to be more deadly than seasonal flu strains as it has apparently caused fatal infection in 50% of the small number of humans so far infected; death rates among infants and the young are particularly high, with one study in Thailand reporting 89% mortality among hospitalized children.[21] Death is usually due to progressive respiratory failure and the acute respiratory distress syndrome, but multi-organ failure can also occur. Although the virus is not efficiently transmitted among humans, ominous signs have appeared since 1977 when the avian epidemic emerged: an increase in viral virulence factors, an enhanced replication capability, and increased resistance to interferons and tumor necrosis factor-α. An expanded host range in avian species, the ability to infect felids, enhanced pathogenicity in experimentally infected mice and ferrets and increased stability in the environment are also worrisome observations, all presaging an evolving and adapting virus that may be primed for a human outbreak. Research institutions and the pharmaceutical industry are working frantically to develop new therapeutics, an H5N1 vaccine, and cell-based vaccine systems that could adapt rapidly to whatever flu strain next emerges. In the midst of all this concern and activity, blood transfusion services have pondered the effect a flu pandemic would have on the blood supply and whether influenza is a blood-transmissible disease.

The evidence for blood transmission of H5N1 is scant and a transfusion-transmitted case has not been reported. An apparent brief viremia makes such transmission possible, and known involvement of the liver and kidney suggests hematogenous spread from the lungs.[22] In the absence of evidence to the contrary, it is safest to assume that avian flu can be blood-transmitted. However, the asymptomatic period of viremia may be very short and the number of blood transmissions small, even if a pandemic occurs. Some plasma components and all commercial plasma fractions will be safe due to inactivation procedures already in place. The larger problem will be disruption of the blood supply engendered by the large number of donors who will be stricken by the disease and perhaps by the quarantine measures that will be invoked. Also, it is estimated that up to one third of blood bank staff will be infected (as will those in transportation systems), putting enormous stress on the whole process of blood collection and delivery. There are no current blood screening measures available, and even if a practical H5N1 nucleic acid assay were developed, it would have little impact: the number of viremic donors will be small, while the number of donors ineligible because of illness and the number of staff unavailable because of infection will be large. The larger problem will be blood delivery and not blood safety.

Prions

Variant Creutzfeld Jakob Disease

vCJD is a new prion disease of man that was first described in the United Kingdom in 1996; it results from consuming tissues from cattle affected by bovine spongiform encephalopathy (BSE), or "mad cow disease." The causative agent is generally accepted to be a prion, a conformational variant of a naturally occurring 33- to 35-kd protein specified by the *PrP* gene. The pathologic protein is protease-resistant and thus is not subject to the normal process of degradation and removal. Furthermore, the pathologic conformer catalyses the same change in other copies of the protein, a process simulating replication. The modified protein forms fibrous aggregates that appear to be involved in the pathogenesis of the disease.

Unlike classic CJD, which is largely (although not exclusively) sporadic in nature, vCJD is etiologically linked with BSE in cattle and its emergence followed the appearance of the cattle disease by 8 to 10 years. At the time of writing, about 161 cases of the human disease have been recognized in the United Kingdom and about 30 cases elsewhere in the world; a number of the latter cases have been attributed to dietary exposure in England. The bovine disease has appeared in many parts of the world, albeit in very much lower numbers than the hundreds of thousand of cases seen in the

United Kingdom. Strenuous efforts have reduced the incidence of cattle disease and, currently, the annual number of human cases is also declining. Unlike for classic CJD, three cases of transmission of vCJD by blood transfusion have been documented by the development of the disease or the detection of the disease agent in patients who were transfused with blood from individuals who subsequently developed vCJD-related dementia and died. All three of the cases occurred in England[23,24] (http://www.hpa.org.uk/hpa/news/articles/press releases/2006/060209.cid.htm). No other secondary routes of transmission are recognized. Efforts have been made to estimate the number of people harboring the infectious prion by assessment of excised tonsils and appendices: in one published study, the agent was found in three of 12,674 tested.[25]

vCJD has a relatively slow onset, characterized by disturbed mentation, dementia, ataxia, myoclonus, and eventual coma and death. Some of the symptoms and the neurophathologic findings differ from those of classic CJD; the onset is usually earlier in life, with most suspicion for cases occurring before the age of 55. The pathophysiology is spongiform degradation of the brain, with characteristic vacuoles and so-called florid plaques. Progression to death is inevitable, usually 9 months to a year after clinical onset. How long the infectious agent is present in the patient before clinical manifestation is unknown, but transfusion transmission has occurred from donations collected as long as 6 years prior to the appearance of frank disease.

There is no definitive pre-mortem diagnostic test, and a preliminary diagnosis may be made on the basis of symptoms and age. Definitive diagnosis depends on pathologic examination of the brain with immunohistochemistry to identify the presence of the protease-resistant prion. Additional testing, particularly for glycoforms, can establish whether the agent is vCJD. No test is available for the detection of infectious prions in the blood of asymptomatic individuals.

The primary intervention in the United States is to indefinitely defer presenting donors with a history of visiting BSE-affected European countries during periods of likely exposure. Individuals who received blood transfusions in the United Kingdom, or who were treated with European-derived bovine insulin, are also deferred. These policies have had a substantial impact upon blood availability in the US. In addition to assay development, there is an ongoing effort to develop affinity filters that may be used to reduce the prion content of red cell concentrates.[26,27]

Other Agents

Many other infectious agents have the potential to be transmitted by transfusion in that they have an asymptomatic blood-borne phase and may survive in blood components during processing and storage. For the past few years there has been some degree of concern about a number of such emerging agents and, in some cases, interventions have been implemented. Unusual visceral presentations of Leishmania infection among troops involved in conflicts in the Persian Gulf countries, along with some evidence of transmissibility, led to temporary deferrals of returnees from Iraq. A potential case of transmission of *Anaplasma phagocytophilum* (the agent of human granulocytic ehrlichosis) has raised further interest in tick-borne infections but has not led to any interventions. In contrast, deferral measures were rapidly implemented during the global outbreak of severe acute respiratory syndromes (SARS), despite the absence of evidence of transfusion-transmissibility. Concern has been expressed about the finding of simian foamy virus (SFV) infection in some animal handlers,[28] as an example of interspecies transmission of a retrovirus—an event thought to reflect the origin of AIDS in human populations. Although lookback studies did not show any transmission of SFV from blood donated by infected animal handlers, and although SFV does not appear to cause disease in humans, regulatory officials have discussed the need for deferral of monkey handlers. Such a policy has been implemented in Canada; the rationale is that interspecies transfer of viruses might be accompanied by mutational events leading to newly pathogenic forms. Genetic variants of existing agents (particularly HIV and hepatitis B virus [HBV]) continue to appear, and such variants may escape detection by current methods. Several agents identified in molecular viral discovery programs have been proposed as the cause of non–A-E hepatitis. These include the closely related flaviviruses, GB virus type C (GBV-C) and hepatitis G virus (HGV), and the new circoviruses, TT virus (TTV) and SEN virus (SENV). Careful clinical and epidemiologic studies have shown that these agents do not cause human hepatitis and, at present, they are agents searching for a disease.[29] Finally, there is continued attention to the possibility that the hemorrhagic fever viruses will be transmissible by transfusion, a concern also extended to additional agents that might be used in bioterrorist attacks.

Pathogen Inactivation

The continued emergence of new infectious agents and of old agents that create emerging problems presents recurrent dilemmas for blood transfusion services. Each new threat raises the need to develop a new donor screening assay or to introduce exclusionary questions into the donor screening history, further marginalizing a limited blood supply. Despite extensive donor testing and detailed donor histories, there remain blood-transmissible agents for which no intervention is in place and the lingering fear that a new agent with the devastating potential of HIV will emerge in the future. One answer to this escalating array of tests and risk factor exclusions is to develop and implement strategies for universal pathogen inactivation that would not only reduce or eliminate the risk of known pathogens but would preemptively destroy any significant pathogen that might emerge in the future.

The concept of physicochemical pathogen reduction has been in development for the past two decades. The greatest success thus far has been in the use of solvent-detergent (S-D) combinations to destroy enveloped viruses, including HIV, human T-lymphotropic virus (HTLV), HBV, and hepatitis C virus (HCV): no transmission of these agents by S-D–treated

plasma derivatives has been reported since this process was implemented. Protection of the commercial plasma supply against the West Nile virus (WNV) epidemic proved the value of a preemptive strategy but also demonstrated the weakness of the S-D method: S-D can only inactivate lipid-enveloped agents and cannot be applied to red cell and platelet products whose function depends on intact lipid membranes. A clear need exists for a broad spectrum inactivation method that could be applied to cellular as well as liquid components of blood. Toward this end, many physicochemical methods to inactivate nucleic acids have been explored. The prized goal of blood safety would be a method to inactivate all viruses, regardless of their nucleic acid structure, as well as bacteria, parasites, and potentially any replicating infectious organism. In addition, the technology should inactivate lymphocytes and prevent fatal transfusion-associated graft-versus-host reactions (only prions would escape because these disease-causing abnormally folded proteins do not contain nucleic acid). Many nucleic acid–inactivating agents have been investigated, most of which depend on the interaction of the effector chemical with an external light source. The system most extensively studied has been the synthetic trimethylpsoralen, designated S-59, in combination with long-wave ultraviolet light. In both plasma and platelet products, this combination inactivates all tested viruses and bacteria, whether DNA or RNA, enveloped or nonenveloped, to levels below the limits of detection, with acceptable maintenance of plasma protein levels and platelet function (but with as much as 20% loss of platelet yield).[30] An alternative to psoralens for the inactivation of infectious agents in platelet products is the vitamin riboflavin (B_2) in the presence of ultraviolet A.[31] This seemingly safer natural vitamin has shown broad inactivation potential but may still reduce platelet survival by 15% to 30%.[32]

One of the major disadvantages to both psoralen and riboflavin is that they cannot be used effectively for the inactivation of red cell products, due to the poor penetration of light into this dense material. Other approaches have been employed to inactivate viruses in red cell products. A frangible anchor-linker-effector compound (FRALE) can crosslink nucleic acid in the absence of light[33]; however, these are alkylating agents that have potential toxicities not only to the blood recipient but also to those handling the blood product. Even if the risk is more theoretic than real, these potential risks may prevent FRALEs and other alkylating agents, such as ethyleneimine (inactines), from being used in the blood bank setting.

In addition to potential toxicity, implementation of viral inactivation strategies into routine blood bank use has been limited because no single method can be used for all blood components and by their high cost. Economic issues can be ameliorated because very effective inactivation would obviate the need for some of our existing blood screening assays, the requirement to introduce new assays for emerging agents, and perhaps also avoid costly leukoreduction. Thus, the relative impracticality and high cost of existing techniques should not deter continued research towards the ideal method that would inactivate nucleic acids in all blood components in a single process and provide an unprecedented level of blood safety, There will never be zero-risk in the blood supply since problems extend beyond infectious disease transmission, but the removal of almost all infectious agents in a single inactivation process is a goal too attractive to abandon without intensive exploration.

Conclusion: Zero-Risk Blood Supply

There continues to be a small but measurable risk of transmission of infectious agents by blood transfusion, even in cases (such as HIV) in which the most aggressive interventions are undertaken. Additionally, as discussed above, emerging agents present new threats to blood safety. Even though the risks of such transmission are almost invariably very much lower than the risks of adverse outcomes from other medical interventions, there is a pervasive pressure to minimize or even eliminate transfusion-transmitted infections. Numerous studies have shown that the marginal costs of such measures far exceed generally accepted norms for cost-effectiveness. Such approaches are driven increasingly by very conservative application of the precautionary principle, without incorporating the usual modifying guidance. A zero-risk blood supply may not be achievable and there is no clear solution to the dilemma of defining acceptable risk. In some cases, resource limitations define the extent of available interventions. In other circumstances the impact of an intervention upon blood availability must be balanced against the gain in safety, as was done in developing deferral criteria in the United States for donor exposure to the risk of exposure to BSE. Finally, even technologies like pathogen reduction, which might offer the elimination of infectivity, may have some low, but tangible toxic risk to patients.

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