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Other emerging viral pathogens

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Introduction

For many years, the greatest concerns about blood safety were focused upon viral infections, most notably hepatitides and retroviral diseases. As a result of experience with these infections, it was widely anticipated that future transfusion-transmissible infections would result from viruses causing persistent infection, most probably parenterally transmitted. However, until around the year 2000, most effort relating to emerging infections was directed towards parasitic diseases. Attention was refocused on viral infections as a result of the outbreak of West Nile virus (WNV) disease in the USA along with the recognition that it was transmissible by transfusion [1]. This paper will briefly review currently emerging viruses other than WNV, which have been shown to be, or which have the potential to be transmitted by transfusion. Specifically omitted are CMV, EBV, hepatitis A virus and the B19 erythrovirus, which are demonstrably transmitted by transfusion, but which do not fit the current definition of emerging agents.

Variant strains and mutants

It should be noted that most RNA viruses and also HBV may mutate at a high frequency as a result of an absence of repair mechanisms during nucleic acid replication. This accounts for the large number of different strains and genotypes that are seen among these viruses, in addition to the pseudotypes that develop in individual infected patients. In the case of HBV, selection pressure can additionally lead to the appearance of so-called escape mutants that fail to express some of the antigens that are normally recognized by neutralizing antibodies [2].

There are a number of different clades of HIV that are broadly grouped as M, O and N. The latter two groups are genetically more distant and, in the case of group O, infection is not always detected by procedures that have been developed to detect antibodies to clade B which was originally most common in the USA and other developed countries [3]. The potential for continuing development of new clades and of recombinant viruses continues, and appropriate surveillance is necessary to ensure that sensitive tests are always available. In the USA, if a test is not validated for the detection of group O strains, donors must be deferred for a history of travel, sexual contact or residence in countries where these strains are found.

Similarly, there are six major genotypes of HCV which are distributed around the world in varying frequencies [4]. Although they may differ somewhat in their pathogenesis and responsiveness to therapy, there do not seem to be the same difficulties in detection as have been seen with HIV [5]. This is also true of the eight genotypes of HBV [6]. However, in addition to HBV genotypes, there are also a number of mutations, some of which result in the absence of effective expression of all of the epitopes, particularly on the common 'a' antigen. Tests constructed with a limited repertoire of monoclonal detection antibodies may be unable to detect some of these mutant strains. Again, however, appropriate surveillance and continuous improvement of test kits usually overcomes these problems.

SARS

Severe acute respiratory syndrome (SARS) is an example of the explosive emergence of an infection apparently new to humans. The disease, in common with most emerging infections, is a zoonosis and it is thought to have been transmitted to humans in south-east China through the preparation of exotic mammals for human consumption, late in 2002 [7,8]. As its name suggests, the disease manifested primarily with respiratory symptoms, but in the more serious cases, was also systemic. It was extremely infectious by the respiratory route, and probably also by the faecal-oral route. As a result of rapid air transportation of infected individuals, it spread rapidly across the globe. Between 1 November 2002 and 10 July 2003, the WHO reported 5910 symptomatic cases, plus another 2527 for which no dates were available. Fortunately, this single outbreak was contained, and apart from a small handful of cases among laboratory workers, there does not appear to have been a recurrence of the disease.

The infectious agent was rapidly identified by molecular techniques as a coronavirus, novel to humans, but endemic among certain mammalian species in south-eastern China [9,10]. It is reasonable to ask why this outbreak of a new respiratory infection led to concern about blood safety. Perhaps the major issues were the seriousness of the disease, including its mortality rate and its remarkably high infectivity.

In addition, as an entirely new disease, nothing was known about it, so preventative measures were taken in accordance with the precautionary principle. The WHO issued recommendations to reduce the risk of transfusion transmission, as did the FDA. These measures included deferral of donors with a history of the disease or contact with a patient and those with a history of travel from an affected area. Even relatively casual travel exposure (such as travel through the airport in Toronto) led to temporary deferral, at least in the USA. Subsequent to the development of these precautions, it became apparent that there could be viraemia during symptomatic disease, but it is still unknown whether there is a presymptomatic viraemia. Additionally, epidemiologic studies showed that there were asymptomatic infections, as judged by the prevalence of antibodies to the SARS coronavirus [11]. Again, however, it is unknown whether such asymptomatic infection is accompanied by viraemia. There were no recorded cases of transfusion-transmission of SARS during the outbreak, although it is unclear whether they would actually have been recognized if they did occur.

HHV–8

The human herpes virus 8 is the most recently recognized herpes virus to infect humans [12]. It is known to be the causative agent of Kaposi's sarcoma, both classical and human immunodeficiency virus–associated, and is the likely causative agent of several other rare disorders, including primary effusion lymphoma and multicentric Castleman's disease [13]. It is naturally transmitted through saliva or sexual contact and has been transmitted through organ transplantation. In some cases, recipients of infected organ transplants have subsequently developed Kaposi's sarcoma [14–16]. Because of the pathogenic potential of this virus and because of the evident potential for blood-borne transmission, there has been a continuing undercurrent of concern about HHV-8 among those involved with blood safety. It is important to note, however, that HHV-8 is not a new agent: rather, it is newly described. Presumably, blood from HHV-8-infected individuals has been transfused for many years, yet there is no extant evidence of an association between transfusion and Kaposi's sarcoma or other HHV-8-associated disease states.

Evidence for transmissibility of HHV-8 by transfusion has been equivocal at best and there continues to be a somewhat anxious wait for the definitive answer to this key question. In 1997, Blackbourn and colleagues [17] reported on the detection of HHV-8 DNA in the blood of a seropositive blood donor; based on evidence of *in vitro* passage of the virus to allogeneic cells, the authors expressed concern about the potential for transmission by transfusion. In the same year, however, Operskalski and colleagues [18] used the TSS repository to examine samples from 10 recipients of blood from 14 donors who were subsequently shown to be HHV-8-seropositive.

None of the recipients showed any evidence of infection. Similarly, negative data on recipients of seropositive blood units have been reported from Jamaica [19]. In contrast, Cannon and associates [20] demonstrated an increased prevalence of HHV-8 antibodies among female injection drug users—a result that was interpreted as evidence favouring blood-borne transmission. Other studies generated similar results. Finally, there have been reports of an increased prevalence of HHV-8 antibodies among some, but not all, groups of blood recipients [21,22]. It is perhaps worth pointing out that there is, as yet, no clear gold standard for HHV-8 antibodies and that the performance characteristics of available tests differ considerably and do not seem to correlate well with the presence of viral DNA [23,24]. False-positive reactions have also been noted.

Dollard and colleagues [25] reported on a study of patient samples from the FACT study, which was a major effort designed to determine the frequency of transfusion-associated infections in a large cohort of cardiac surgery patients from 1986 to 1990. Among the 284 patients who were initially seronegative, 2 had unequivocal increases in HHV-8 antibody titres 6 months postoperatively. This translated to a risk of 0·082% per unit transfused. Although there were no infections among a control group of 75 non-transfused individuals, the difference was not significant. The overall frequency of existing positive test results in the study population was quite high, at 11·3%, although, in a different study, the donor prevalence was found to be 2·4%. As with other studies, there is no clear donor-recipient linkage or real proof that transfusion-associated transmission had occurred. Linked donor samples were not available. The study adds to the weight of circumstantial evidence, however, that HHV-8 may be transmissible via transfusion. More convincing evidence of transmissibility has been developed in a study in Uganda, and was recently presented in preliminary form.

Given the variable behaviour characteristics of tests for HHV-8 antibodies, and the absence of clear evidence that DNA testing would be effective, it is unclear how interventions for this infection will be implemented, if needed. It is, however, quite likely that leucoreduction will be able to effect a substantial, if not complete, abrogation of infectivity.

Dengue

Dengue is caused by a mosquito-borne flavivirus which is endemic in most of the tropics. It has the potential for emergence or re-emergence in areas where the vector mosquitoes exist, including the south-eastern USA. By way of example, an outbreak of dengue has occurred in tropical Queensland, Australia; it was thought to have been introduced by infected travellers. Although infection with dengue virus is usually acute, the example of WNV shows that this is not necessarily a barrier to transmission by transfusion. However, given the

very high incidence of dengue, it is perhaps surprising that more cases of transfusion-transmitted disease have not been reported. In fact, there has been one well-documented case reported in Hong Kong, plus a potential case in a bone marrow transplant recipient in Puerto Rico. It seems likely that the infectious viraemic period must be very short, and/or that cases are not readily recognized as transfusion-transmitted in an epidemic environment.

A complication of epidemic dengue for transfusionists is the fact that, because there are four circulating strains of the virus, patients may suffer a second infection. In such cases, an even more serious disease, dengue haemorrhagic fever may result [26]. This disease state often requires platelet transfusions to correct bleeding, thus further stressing transfusion services.

Investigations are currently under way to determine the extent of dengue viraemia among blood donors in epidemic areas, such as Brazil and Puerto Rico. This information will help to establish the potential risk of transmission and indicate the extent to which a testing strategy would be effective.

Pandemic influenza

There is considerable current concern about the possibility of an influenza pandemic. This concern is based upon the historical fact that there have been periodic pandemics associated with the circulation of new strains of the virus in humans and the current outbreak of the H5N1 strain of avian influenza, which causes high mortality when it does infect humans [27]. In addition, other strains of avian influenza viruses are also circulating. While there is no evidence that H5N1 will (or will not) result in a human pandemic, its presence has triggered significant levels of pandemic preparedness.

As with the SARS virus, there is evidence that H5N1 will result in viraemia during symptomatic infection, but it is unknown whether there is a presymptomatic or asymptomatic viraemia. Thus, it is unclear whether there will be a risk to blood safety during an influenza pandemic. Consequently, it is unclear whether donors would need to be deferred after disease, or after contact with infected individuals. There do not appear to be any documented cases of transfusion transmission of influenza during normal periodic outbreaks, but it is unclear whether such a transmission would actually be recognized. It is also unclear how an intravenously inoculated infection would be manifested.

What is clear is that an influenza pandemic would have a profound effect upon the ability of the medical system to provide an adequate supply of blood in the face of widespread sickness among donors and blood centre staff. Additionally, it is not entirely clear how a pandemic would impact the needs for blood.

HEV

Hepatitis E virus (HEV) causes an epidemic form of hepatitis that is self-limited [28]. The disease is somewhat similar to hepatitis A, although it is much more severe in pregnancy. Transmission is by the faecal–oral route and is most often water-borne. The virus is related to the calicivirus group and, as such, is a non-enveloped virus that has an RNA genome. Although there is some evidence (based largely on seroprevalence studies) that HEV is present in the USA, it is found predominantly in tropical countries. Indeed, most cases identified in the USA appear to have resulted from infections that occurred in countries where HEV is endemic. HEV infection is self-limited and acute and it has generally been thought that there is little risk of transmission by transfusion. Until recently, no cases of transfusion-associated HEV infection of disease have been reported [29]. However, a number of recent cases of such transmission have been reported from non-endemic areas, emphasizing that acute infections are also transmissible by transfusion, as long as there is an asymptomatic viraemic phase [30,31]. This has, of course, been abundantly illustrated by the example of West Nile virus in the USA [1].

GBV–C/HGV, SEN–V

There has been a continuing search for additional hepatitis viruses and, over the past few years, two different viruses or virus groups have been identified in this context. The first of such putative hepatitis viruses was identified by two separate groups in the late 1990s. In one case, scientists at Abbott Laboratories looked for genomic sequences related to those of an existing isolate known as the GB virus (GBV), which had previously been associated with hepatitis in a physician. Three viruses were identified, one of which (termed GBV-C) was found among a number of human sources [32]. Working in parallel, but using a different approach, scientists at Gene-Laboratories isolated viral RNA sequences and characterized a virus they termed hepatitis G virus (HGV) [33]. It is generally accepted that these two isolates were, in fact, representatives of essentially the same virus group, which is now known as HGV.

HGV, like HCV, appears to be closely related to the flavivirus group. It is found among a relatively high proportion of the normal population, as exemplified by blood donors. Its presence has been demonstrated both by seroprevalence studies, in which the frequency of antibodies is 3% to 15%, and more interestingly by detection of viral RNA in the plasma of 1% to 3% of normal subjects [34]. Perhaps not surprisingly, the virus is readily transmissible by transfusion and is found at high prevalence among individuals who have undergone multiple transfusions. However, it has not proved possible to demonstrate that infection with HGV is associated

with hepatitis or even with signs of mild liver disease, such as elevated ALT levels. Indeed, HGV appears to be a virus that is currently in search of a disease. The term 'hepatitis' in its name may be a misnomer, attributable only to the fact that the virus was found in association with hepatitis in the first place. It is also important to recognise that the worldwide distribution of HGV clearly shows that it is not a new virus but, rather, one that has coexisted with humans for many centuries.

There have, however, been some intriguing observations that clearly suggest that HGV/GBV-C may have an impact on the course of HIV disease. For example, studies have shown lower mortality among co-infected individuals relative to those with HIV only [35,36]. The mechanism for this effect is unclear, but may be due to the effects of infection on the levels of a number of chemokines [37].

Curiously, another two viruses, TTV and SEN-V, were separately identified among individuals with hepatitis and were also shown to be poorly, if at all, associated with hepatitis. These viruses were also found to cause prolonged viraemia and, in some cases, turned out to be present in up to 90% of the population. Like HGV, they were readily transmitted by transfusion. Both viruses were thought to be representatives of the circovirus group: small, non-enveloped viruses with a circular DNA genome. This group of viruses had not previously been described among humans.

Workers in Japan used representational difference analysis to isolate DNA sequences from three patients with unexplained post-transfusion hepatitis. The sequence was established as viral, and the virus was named TTV, reflecting the initials of the patient from whom it was isolated [38]. A considerable amount of research has revealed some of the key features of this agent [39]. It is a small virus with a covalently closed, circular DNA genome of around 3800 bases. The virus is thought to be non-enveloped and is most closely related to the circoviruses, which are responsible for a number of diseases in plants and a handful of mammalian or avian disease states. The classification of TTV is currently incomplete, and proposals have been made to place it in at least two genera. What has become apparent is that TTV is a member of an extremely diverse group of viruses, as demonstrated by considerable variation in genomic sequence.

Epidemiologic studies have confirmed that TTV is a widely distributed virus and have clearly established that it is transmissible by transfusion. Interestingly, it also appears to be transmitted by the vertical, faecal–oral, and, perhaps, other routes.

A key issue is the clinical significance of this group of viruses. Although the original source of the virus and its apparent association with ALT elevations implied that TTV was indeed a hepatitis virus, this identification no longer seems tenable. Indeed, there are far more infections without ALT elevations than with such evidence of liver disease. Even

in clear, transfusion-associated transmission of TTV, the recipients did not manifest ALT elevations in any pattern that could be associated with the infection. Thus, at this stage, there is little evidence that this virus expresses any pathogenic potential. However, it is certainly too early to conclude that this entire group of viruses is without any clinical significance.

After the recognition of TTV, the search for hepatitis viruses continued, and Primi and colleagues [39,40] used degenerate primers derived from TTV to probe samples from selected patients. An isolate was identified and named SEN-V (after the initials of the source patient, an HIV-infected injection drug user). Eventually, at least eight different strains were isolated, termed A through H. The SEN group has been shown to be one branch of the TTV group, seeming to share its epidemiologic characteristics. Although two of the strains (D and H) have been associated with evidence of transfusion-associated hepatitis, a causal relationship has not been established [40,41].

Other viruses of interest

Over the past few years, there have been a number of unexpected cases of transmission of infectious agents through organ transplantation. In some cases, such transmissions could imply that the agents may be transmissible by blood, which is certainly true of *Trypanosoma cruzi*, a protozoan parasite that has been involved in at least three clusters of transplant-associated transmission. Two viruses have been similarly involved, although it must be acknowledged that neither are currently considered to be emerging pathogens. There have been two well-documented cases of transmission of rabies by organ transplantation [42,43], plus one cluster of transmissions of lymphocytic choriomeningitis virus (LCMV) [44].

Rabies virus infection is not thought to result in viraemia and it was considered likely that the transmission resulted from infection of, and via the sympathetic innervation of the transplanted organs. On the other hand, LCMV may generate high levels of viraemia in its normal rodent hosts and viraemia was likely in the organ donor, who was shown to have acquired the infection from a pet hamster. There have, however, been no reports of transfusion transmission of either rabies virus or LCMV. At this time, no specific interventions are recommended for blood donors who may have been exposed to either infection.

Concern has been expressed about the potential for transmission of simian foamy virus (SFV), a retrovirus. It has been found to infect a small number of animal handlers and it has been theorized that such individuals might be able to transmit the virus through transfusion of their blood. However, foamy viruses seem to be apathogenic, both in their natural hosts and in humans. The underlying concern is that viruses that jump species may turn out to be highly pathogenic in the new host. Additionally, as discussed above, many retroviruses are

prone to frequent mutation, so that pathogenic strains could emerge. The issue has been discussed by regulatory authorities in the USA, and to date, not action has been taken. However, in Canada, animal handlers who have frequent contact with primates are deferred. One report details look-back to recipients of blood from an animal handler infected with SFV, but no recipient tested had any evidence of infection with the virus [45].

At the time of writing, there has been an unexpected outbreak of mumps in the midwestern states of the USA and previously in the UK. Although there have been no recorded cases of transmission of mumps virus by transfusion, it is known that there is a viraemic phase and modest precautions to reduce the theoretical risk of transmission have been recommended by the AABB in the USA.

Discussion

In the developed world, the major transfusion-transmitted agents (with the exception of syphilis) have traditionally been viral. Interestingly, the general expectation at the end of the 20th century was that the next threat to blood safety would turn out to be a persistent, sexually transmitted virus. However, this was not the case. The unexpected emergence of WNV and its transmissibility by transfusion has taught us to broaden our thinking around the concept of transfusion-associated infection. Nevertheless there are relatively few emerging viral infections that have shown any evidence of transmission by this route. There are certainly numerous viruses that have the potential for such transmission and continued vigilance is necessary. There are, however, no clear patterns that will define appropriate target patient groups for surveillance and we will have to continue to rely upon haemovigilance systems and astute clinicians to identify cases of emerging viral infections among blood recipients. Careful and appropriate use of the precautionary principle should also help to maintain the safety of the blood supply.

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