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研究報告の概要

問題点（現在の米国での血液供給の安全性）

米国で実施している血液製剤の安全対策に関する総説論文である。米国において、献血ドナーを注意深く選択し、スクリーニングの問診、NATなどの各種ウイルスのテストを実施した結果、HIVとHCV感染は献血1500万件ついて1件に減少している。
しかしながら、新興感染症であるシャーガス病、バベシア症、マラリア、WNVとvCJDに注意しなければならない。
バベシア症は、ダニによる感染である。米国で輸血によるバベシア症の感染は約50件の報告がある。日本は1件の報告がある。献血時のスクリーニングテストはなく、問診等も効果がない。輸血感染による致命的な症例はないが、高齢者、免疫低下患者、無脾症患者は危険性がある。
マラリアは、米国で年間1-2例の輸血感染がある。献血時の問診で渡航歴の危険性の高いドナーは排除しているが、最近の感染例はこの問診時に問題があったためである。
シャーガス病は、ラテンアメリカでは一般的な寄生虫(T. cruzi)疾患であり、米国で4件、カナダで2件の確定した感染報告がある。現在米国でT. cruzi抗体のテストはない。献血時にシャーガス病であるか問診されるだけである。臨床研究によると、輸血を受けた総計120,000人の心臓手術患者でT. cruziが感染した例はない。
WNVのNAT導入により、約1000件のWNV-RNA陽性の献血ドナーを見つけ、感染の伝播を防いでいる。WNVの低titerやミニプールNAT検出レベル以下の場合、検出できず感染する恐れがあるが、確定された感染例はない。

使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
採血国である米国の血液供給を脅かす情報を入手した為、報告する。 シャーガス病は、T. cruziによる感染症で、大きさが約20ミクロンであり、本剤を含む弊社の血漿分画製剤における製造工程、特に滅菌ろ過(0.22ミクロン等)で十分除去できるものである。また輸血からの感染報告は存在するが、血漿分画製剤からの感染の報告はない。また63℃、30分の加熱で死滅する報告がある。 マラリアも同様に、血液製剤の製造過程で寄生虫は除去される報告がある。 シャーガス病、マラリア、バベシア症に関して、弊社の血漿分画製剤は滅菌濾過等により安全である。 WNVも、製造工程中で60℃10時間の液状加熱で不活化されるので、本剤では特に問題ないと考える。	今後ともvCJDなどの新興感染症に関する情報収集に努める所存である。

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Current Safety of the Blood Supply in the United States

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Abstract

In common with other developed countries, the United States has placed a great deal of emphasis on blood safety. As a result of careful donor selection and the use of advanced tests, including nucleic acid testing (NAT), the risk of transmission of human immunodeficiency virus and hepatitis C virus has been reduced to about 1 in 1.5 million donations. NAT for hepatitis B virus has not been introduced, but nevertheless the risk is low. Attention recently has been focused on emerging infections. NAT for West Nile virus was implemented within 6 to 8 months of recognition of the need to prevent transfusion transmission of this newly introduced virus. Approximately 1000 potentially infectious donations were identified and removed from the blood supply during the 2003 season. Other emerging infections attracting attention include Chagas' disease, babesiosis, malaria, and variant Creutzfeldt-Jakob disease.

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Key words: Blood transfusion; HIV; Hepatitis C; Hepatitis B; Emerging infections

1. Introduction

In the United States, blood safety depends on selection of voluntary donors, extensive use of screening questions, laboratory testing, and maintenance of deferral registries. These processes are highly regulated and are managed under voluntary quality systems such as the standards of the American Association of Blood Banks (AABB). Over the years, there has been a process of continuing improvement, particularly in testing. This process has resulted in a very low frequency of residual infectivity from the blood supply, at least for hepatitis and retroviral infections. The recent introduction of nucleic acid testing (NAT) has had a major impact on safety [1-3]. At the same time, a number of new threats to blood safety have appeared and necessitated additional donor deferral and/or testing measures [4]. Notable among these new infections have been West Nile virus (WNV) and variant Creutzfeldt-Jakob disease (vCJD).

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2. Current Risk of Hepatitis Viruses and Retroviruses

The original approach to controlling transfusion-transmitted hepatitis and, later, acquired immunodeficiency syndrome (AIDS) involved careful questioning of donors about their medical history and risk behaviors. The majority of these questions are still in place, despite the use of tests of increasing sensitivity. Overall, however, very few donors are deferred as a result of these questions, but there is good evidence that almost 2% of donors may fail to report deferrable risk behaviors during the donation process [5]. Nevertheless, both the prevalence and incidence of human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV) infection among donors is much lower than that attributable to the general population (Table 1) [6]. It seems likely that the majority of infected and at-risk individuals do not donate as a result of the use of a voluntary donor population along with broad public education.

In the United States, the following tests are performed on all blood donations: antibodies to hepatitis B core antigen (anti-HBc), hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (anti-HCV), antibodies to HIV-1 and HIV-2 (anti–HIV-1/2), antibodies to human T-lymphotrophic virus I (HTLV-I) and HTLV-II (anti–HTLV-I/II), serologic test for syphilis, and minipool NAT for HIV and HCV RNA. In addition, all donations are tested by investigational NAT

Table 1.
Prevalence and Incidence of Major Transfusion-Transmissible Infections among the US Population and among US Voluntary Blood Donors*

	Prevalence, pht		Incidence, phtpy	
Infection	Population	Donors	Population	Donors
HBV (HBsAg)	420	77	27.9	1.3
HCV	1800	304	8.9	1.9
HIV	200	10	14.3	1.6

*From [1,6]. pht indicates per hundred thousand; phtpy, per hundred thousand person-years; HBV, hepatitis B virus; HBsAg, hepatitis B surface antigen; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

for WNV RNA (see later). Despite the use of these tests, posttransfusion infections continue to be reported [7-9]. Essentially all of them have been identified as a result of focused lookback procedures. The frequency of these occurrences is very low, but the efficacy of reporting is questionable. Consequently, the risk of transfusion-transmitted infections is usually projected from evaluation of donor testing results.

A number of circumstances may contribute to posttransfusion infections, even when laboratory tests are used. These circumstances include laboratory error, infections with variant or mutant viruses that evade detection, and collection of blood during the window period. Busch analyzed the relative contribution of each of these factors and concluded that the major risk of posttransfusion infection is collection of blood during the test-negative window period [10].

The risk of residual infection from window-period donations can be calculated by simply multiplying the length of the window period by the incidence of new infection in the donor population. The window period for key transmissible infections has been defined either by direct observation of the period between exposure and development of the infection marker or by calculation from lookback cases. The window period estimates have been continuously corrected to reflect the use of tests of increasing sensitivity. The assessment of the effect of such tests has been done by examining closely spaced samples collected during seroconversion. Such panels of samples have been obtained from plasmapheresis donations.

The primary means of defining incidence rates among donors has been by direct observation of the frequency of new infections in large populations of regular donors. Thus many estimates of residual risk are applicable only to repeat donors. However, the incidence rates for infections among first-time donors also have been measured by careful use of modified or additional tests. For example, Janssen and colleagues used a less-sensitive anti-HIV test to measure the proportion of HIV-positive donors who were in the first 3 months of infection [11]. These observations allowed direct measurement of incidence. Somewhat similarly, Dodd and colleagues evaluated the frequency of donations that were positive for HCV RNA but negative for anti-HCV among first time donors [1]. Knowledge of the length of the RNA-only period again allowed estimation of the incidence rate for HCV. It was interesting that both of these studies showed that in US donor populations, the incidence of new infections among first-time donors is about 2.4-fold greater than that among repeat donors.

Table 2 shows an estimate of residual risk of retroviruses and hepatitis viruses among voluntary blood donors in the United States, both for repeat donors and for the overall donor population, which includes approximately 22.8% donations from first-time donors. In some cases (HBV and HTLV), there is no direct measure of incidence among first-time donors, so it has been assumed that the frequency is 2.5-fold greater than that for repeat donors. The impact of NAT also is shown in Table 2.

Since 1999, all donations in the United States have been tested by nucleic acid amplification methods for the presence of HCV and HIV RNA. Two commercial methods are in use, and most testing is performed on minipools of 16 or 24 samples, depending on the selected method. The yield of RNA-positive, antibody-negative samples has been 1 in 230,000 for HCV and 1 in 3.1 million for HIV [2]. As Table 2 shows, the impact of removing these donations from the blood supply has been reflected in an appreciable increment in blood safety. At the time of this writing, procedures for detecting HBV DNA in minipools have been evaluated, and data have been submitted to the US Food and Drug Administration in support of applications for licensure of these procedures. In at least one study, a small number of HBV DNA–positive yield samples were detected, but it is anticipated that HBsAg tests of increased sensitivity will offer a similar safety increment to DNA testing in minipools.

3. West Nile Virus

WNV is a good example of the unexpected emergence of an existing virus in a previously uninfected geographic locale. WNV is a flavivirus of the Japanese encephalitis serogroup. It is spread mainly by mosquitoes and, until the outbreak in the Americas, was endemic only in southern Europe, parts of Africa, and the Middle East. In these locations, the virus seems to be largely in equilibrium, although there have been a number of localized outbreaks of human disease. In these areas, the virus had never been considered to offer any threat to blood safety.

In 1999, WNV appeared for the first time in North America, in the form of a small but intense outbreak in New York City with 66 recognized human cases and 2 deaths. In the

Table 2.
Estimated Residual Risk of Infection from the American Red Cross Voluntary Blood Supply*

	Residual Risk from	
Infection	Repeat Donors	All Donors
HBV	1:205,000	1:144,000
HCV without NAT	1:276,000	1:199,000
HCV with NAT	1:1,935,000	1:1,390,000
HIV without NAT	1:1,468,000	1:1,048,000
HIV with NAT	1:2,135,000	1;1,525,000
HTLV	1:2,993,000	1:1,208,000

*Adapted from [1]. HBV indicates hepatitis B virus; HCV, hepatitis C virus; NAT, nucleic acid testing; HIV, human immunodeficiency virus; HTLV, human T-lymphotrophic virus.

next 2 years, a similar number of cases were seen, but surveillance studies revealed that the virus was spreading to a larger area each year. The major amplifying hosts were a number of bird species, and significant avian mortality occurred. At that stage in the epidemic, there was little concern about the risk of transfusion transmission of WNV, although a risk estimate was published for the initial outbreak [12]. In 2002, however, there was an enormous outbreak of human cases, totaling 4156 with 284 deaths and affecting the majority of the continental United States. Of most concern, 61 potential cases of transfusion-transmitted WNV infections were reported, and of these 23 were confirmed [13]. In all cases in which samples of the implicated donations were available, it was found that readily detectable levels of WNV RNA were present.

These observations led to rapid development and implementation of NAT for WNV. The decision to move toward such testing was made in September 2002, and tests were fully in place before the start of the 2003 WNV season in July. During 2003 there were 9388 human cases of WNV disease with 246 deaths. The cases occurred over an even broader geographical area than that affected in 2002. Blood collectors identified approximately 1000 WNV RNA–positive donations (a rate of about 1 in 5000), preventing many potential infections among blood recipients. In some localities, the frequency of finding RNA-positive donors was extremely high (for example, 1 in 47 in parts of Nebraska) [14]. As a result of concerns that NAT in minipools did not detect all RNA-positive donations and the eventual demonstration of recipient infections attributable to such low-titer samples, limited single-donation testing was implemented in some areas of highest incidence of WNV infection. It was clear that this approach did detect some otherwise undetectable, potentially infectious samples. The practice of performing resource-limited single-donation testing of this type continued into the WNV season in 2004. During 2003, 6 confirmed cases of transfusion-transmitted WNV were reported. All seemed to be attributable to donations with very low titers of WNV, below the levels detectable by minipool testing. As of this writing, however, there has been no authenticated case in which transmission has been attributable to a blood unit with detectable levels of WNV immunoglobulin M (IgM), although it is known that IgM and WNV RNA may coexist for a time. Thus the unexpected emergence of WNV and the finding of its transmissibility by transfusion posed a significant challenge in 2002 [15]. Rapid development and implementation of NAT clearly had a significant impact on the problem, although it has not proven possible to entirely eliminate the risk.

4. Other Infections

4.1. Syphilis

All donations are tested for syphilis with treponemal tests, nontreponemal tests, or both. There has been no reported case of transfusion syphilis in the United States for well over 40 years. It is possible that this outcome is a result of continuing testing, and it has not proven possible to eliminate the requirement for such testing [16]. In recent studies, however, Orton and her colleagues did not find treponemal DNA and/or RNA in 169 blood donor samples with confirmed positive serological test results for syphilis [17]. Thus the potential for detection of an infectious sample appears to be low.

4.2. Malaria

Malaria is probably the infection most frequently transmitted by transfusion. However, such transmission is a rarity in the United States with only 1 or 2 cases annually [18]. Approximately 1000 cases of imported malaria are diagnosed each year in the United States. This number is small compared with the numbers in, for example, Western Europe. There is a comprehensive effort to exclude at-risk donors by careful questioning about their travel history. Many of the recent cases of transfusion transmission of malaria appear to be attributable to failures in the questioning process. Although endemic malaria has been eliminated from the United States, there is concern about the occurrence of epidemiologically unexplainable cases, most recently in Virginia and Florida. At least some of such cases are attributable to mosquito-borne transmission from migrant workers or travelers, but it is clear that a questioning strategy would be ineffective in identifying such secondary cases if the individuals were to present to give blood. This is a situation that deserves future scrutiny.

4.3. Chagas' Disease

It is well-established that Chagas' disease (caused by the protozoan parasite *Trypanosoma cruzi*) is transmitted by blood transfusion. In Latin America, where human infection is endemic, it is estimated that a recipient of parasitemic blood has a 12% to 50% chance of being infected. Because infection is often lifelong, population movements from endemic areas lead to the presence of infected and potentially infectious individuals in nonendemic areas such as the United States. There have been a total of 6 well-authenticated transfusion transmitted cases of *T cruzi* infection in the United States (4 cases) and Canada (2 cases) [4]. These cases are thought to be a substantial minority of the cases that might occur, because the disease is not readily diagnosed, nor is it often suspected. One of the recognized cases was identified only as a result of careful follow-up of a patient inadvertently given a transfusion of seropositive platelets [19]. Essentially all cases were traced to donors who had been infected early in life in areas of endemicity. There is currently no testing for *T cruzi* antibodies in the United States, and donors are asked only if they have had Chagas' disease. This measure is very insensitive [20]. Seroprevalence studies have shown that in areas with a high proportion of migrants from Latin America, as many as approximately 1 in 7500 donors may be in the seropositive state, and approximately 60% of these donors actually have parasitemia, as demonstrated by polymerase chain reaction analysis and or parasite culture [4]. It is thought that the national seroprevalence rate may be between 1 in 40,000 and 1 in 25,000, suggesting a potential for a few hundred infections each year. Lookback studies, however, did not identify any infected recipients within a group of 19 patients who received blood

from donors subsequently found to have seropositive results. A study of cardiac surgery patients receiving a total of approximately 120,000 transfusion products did not identify any new transmissions of *T cruzi* [21]. It is possible that in an environment in which component therapy is the rule, most transmissions actually come from platelets rather than red blood cells.

4.4. Babesiosis

Babesia organisms are small, intraerythrocytic protozoan parasites. Their natural hosts are mammals, and they are transmitted by ticks. Humans are accidentally infected as a result of a bite from an infected tick. In the United States, the predominant species is *Babesia microti*; which is endemic in the Northeast coastal area and in parts of the upper Midwest (Wisconsin and Minnesota). Other species have been found on the West Coast and in Missouri and Kentucky. Approximately 50 cases of transfusion-transmitted babesiosis have been reported in the United States. However, seroprevalence studies show that approximately 1% of blood donors are antibody-positive in endemic areas of Connecticut and as many as 60% of these individuals may have parasitemia and transmit the infection to recipients of their blood. The overall infection risk in Connecticut has been estimated at 1 per 1800 units of red cell concentrates [4]. It is interesting that there has been a single report of transfusion transmission of *Babesia* organisms in Japan [22]. No routine test is currently available, and epidemiologic or risk questions are ineffective in identifying infectious donors. In most cases, the disease is readily treatable. However, some fatal transfusion-transmitted cases have occurred. Elderly, immunocompromised, and asplenic patients are at most risk.

4.5. Transmissible Spongiform Encephalopathies

Transmissible spongiform encephalopathies (TSEs) are progressive, uniformly fatal, degenerative neurologic diseases that occur in mammals and humans. The prototypic human TSE is Creutzfeldt-Jakob disease (CJD), which occurs with a worldwide annual incidence of about 1 per 1 million. The TSEs are thought to be caused by prions, or conformational variants of a normal protein. It is thought that the pathologic form of the prion can catalyze the same conformational change in the normal protein, thus replicating the infectious agent. The prions are themselves infectious, and CJD has been iatrogenically transmitted by the use of neurosurgical instruments, by transplantation of dura mater prepared from infected donors, and by the use of pituitary-derived human growth hormone. A particular feature of the infectious agent of TSEs is its resistance to inactivation. For many years, there has been concern that CJD might be transmissible by blood transfusion, but at the time of this writing, there has been no evidence of such transmission.

Over the past 15 years, a new TSE, bovine spongiform encephalopathy, has emerged among cattle, particularly in the United Kingdom, where hundreds of thousands of cases occurred [23,24]. The disease also has been reported from many countries and has a global distribution, probably as a result of export of cattle feed and live cattle. The disease arose as a result of a policy of feeding meat and bone meal to cattle, allowing recirculation of the infectious agent. The infectious agent has been transferred to human populations as a result of consumption of meat and meat products from affected animals. The resulting human disease is a clinically and pathologically distinct TSE termed variant CJD (vCJD) [25]. As of the middle of 2004, almost 150 deaths had been attributed to vCJD, the majority of which occurred in the United Kingdom. Because of its natural history, distribution in the body, and results of animal model studies, it was feared that vCJD might be transmissible by transfusion. At the time of this writing, 2 likely cases of such transmission have been reported, both in the United Kingdom [26,27]. As of this writing, it is not possible to characterize the actual risk of further cases, although the risk is likely to be very low. A number of precautionary measures have been taken to reduce the risk. In particular, in countries outside the United Kingdom, persons who have spent time in the United Kingdom or in other European countries are deferred from donation.

4.6. Bacteria

The most frequent microbial adverse events in transfusion medicine are septic reactions due to bacterial contamination of platelet products. A US national surveillance study showed a rate of confirmed septic events of approximately 10 per 1 million platelet transfusions, and approximately 20% of these events were fatal [28]. In contrast, a prolonged study in a single hospital showed a frequency of patient reactions of approximately 1 per 15,000 units transfused [29]. Although it was generally accepted that the risk of such reactions increased with the length of storage (which is up to 5 days in the United States), the national study reported that deaths occurred at a median of 2.5 days of storage, perhaps as a result of the presence of fast-growing bacteria [28]. In a number of countries, procedures to evaluate platelet contamination by automated culture have been implemented. In the United States, in March of 2004 the AABB started requiring methods for limiting and detecting bacteria in platelets. Almost all apheresis platelets are evaluated by automated culture, but this practice has not proved possible for whole blood–derived platelets, which are generally evaluated by surrogate methods with lesser sensitivity. Overall, apheresis platelets are yielding reactive culture results at a rate of about 1 per 2000, but one half or more of such findings are falsely positive. It seems likely that these measures will prevent the transfusion of at least a proportion of bacteria-contaminated platelets.

5. Summary

Overall, the risk of infection from blood transfusion is very low in the United States, significantly less than 1 in 1 million for HCV, HIV, and HTLV. The risk of HBV infection may be somewhat higher, although there are essentially no contemporary reports of posttransfusion hepatitis B. In contrast, it appears that the risk of infection by other agents (particularly certain parasites) may be much greater. It has

been shown that a rapid response to a newly emerging, transfusion-transmissible agent is possible, as in the case of WNV.

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医薬品
医薬部外品　研究報告　調査報告書
化粧品

識別番号・報告回数		回	報告日 年　月　日	第一報入手日 2005 年 2 月 9 日	新医薬品等の区分 該当なし	厚生労働省処理欄
一般的名称			研究報告の公表状況	Inactivation of parvovirus B19 by liquid heating incorporated in the manufac- turing process of human intravenous Immunoglobulin preparations Yunoki et al., Brit. J. Haematol, 2004;128: 401-404	公表国 英国	
販売名（企業名）						
研究報告の概要	各種熱不活化処理を施した血漿分画製剤の投与によるヒトパルボウィルス B19 の感染の可能性を示唆する文献がこれまでにいくつか報告されている。本研究では，静注用人免疫グロブリン製剤の製造工程で導入されている液状加熱処理(60℃，10 時間)によるパルボウイルス B19 の除去能について検討された。その結果，パルボウイルス B19 は同条件下で迅速に不活化されることが示された。これに対し，従来ヒトパルボウイルス B19 の実験モデルとして用いられてきたイヌパルボウィルス(CPV)の感染性は，液状加熱処理の影響をほとんど受けなかった。					使用上の注意記載状況・その他参考事項等

報告企業の意見	今後の対応
本研究報告は，ヒトパルボウイルス B19 に相当すると考えられていたモデルウイルスとは異なり，製造工程中の 60℃10 時間の熱処理によりヒトパルボウイルス B19 が迅速に不活化され得ることを示唆するものである。弊社の血漿分画製剤およびフェリチン(コージネイト FS の製造工程で使用)の製造工程でも同様の加熱処理が取り入れられている。ヒト免疫グロブリン製剤については加熱処理が行われていないが，ヒトパルボウイルス B19 モデルを用いた試験で，血漿分画ろ過工程において除去能が確認されている。	現時点で新たな安全対策上の措置を講じる必要は無いと考える。引き続き関連情報の収集に努める。

bjh research paper

Inactivation of parvovirus B19 by liquid heating incorporated in the manufacturing process of human intravenous immunoglobulin preparations

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Summary

Several reports have suggested the possible transmission of human parvovirus B19 (B19) through the administration of plasma derivatives that had undergone virus inactivation by various types of heat treatment. However, none of the reports evaluated and discussed the inactivation of B19 by the heat treatment that is implemented in the individual manufacturing processes of such products. The present study evaluated the ability to inactivate B19 of liquid-heat treatment at 60°C for 10 h that was incorporated in the manufacturing process of intravenous human immunoglobulin preparations. The results showed that B19 was rapidly inactivated under the conditions used for the liquid-heat treatment.

Keywords: parvovirus B19, intravenous immunoglobulin, viral safety, virus inactivation, transmission.

Human parvovirus B19 (B19) is not so pathogenic to healthy individuals; however, this virus does present a certain level of risk, because it may cause serious pathological conditions in pregnant women and immunosuppressed individuals. As B19 has no envelope, it is resistant to detergent- or organic-solvent treatment. In addition, B19 is a small virus (diameter, 18–26 nm), which means that it is not easy to eliminate by filtration through virus removal membranes. Liquid-heat treatment, which is considered to be a highly reliable method for virus inactivation, has been introduced in the manufacturing processes of various plasma derivatives. Investigation using model viruses shows that B19 is heat resistant, suggesting that the evaluation of the obtained results for inactivation may be limited (The European Agency for the Evaluation of Medicinal Products/Committee for Proprietary Medical Products (CPMP) Biotechnology Working Party, 2001a; The European Agency for the Evaluation of Medicinal Products/Committee for Proprietary Medical Products (CPMP) Biotechnology Working Party, 2003).

To date, the heat stability of B19 has been based on the heat sensitivity of porcine parvovirus (PPV) and canine parvovirus (CPV), used as model viruses, and epidemiologic information such as case reports on seroconversion after administration of plasma derivatives. In fact, these model viruses have been widely used to evaluate safety of the plasma derivatives according to the guidelines (The European Agency for the Evaluation of Medicinal Products/Committee for Proprietary Medical Products (CPMP) Biotechnology Working Party, 1996, 2001b; The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH), 1997; Pharmaceutical and Food Safety Bureau, Ministry of Health, Labour and Welfare, 1999).

Recent reports have shown that under liquid-heating conditions, B19, unlike other animal parvoviruses, was heat sensitive (Blümel *et al*, 2002a; Yunoki *et al*, 2003). The reports suggested that, under the heating conditions employed in manufacturing process of albumin preparations, B19 should rapidly be inactivated and that its heat sensitivity should differ depending on the specific stabilizer used in the inactivation treatment (Yunoki *et al*, 2003). The present study evaluated the heat sensitivity of B19 under the liquid-heat treatment conditions currently implemented in the manufacturing process of intravenous immunoglobulin (IVIG), polyethylene glycol (PEG)-treated/heat-treated preparations and demonstrated that the virus was rapidly inactivated under the conditions used.

Materials and methods

IVIG heat-treated/PEG-treated preparation (heat/PEG-treated IVIG).

Heat/PEG-treated IVIG preparations, derived from Japanese donors (Kenketsu Venoglobulin®-IH, Benesis Corp., Osaka,

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Japan) or US donors (Venoglobulin®-IH, Benesis Corp.), were used in this study. In order to exclude interference by neutralizing antibody to the infectivity assay system of B19, a sample was prepared immediately before heat treatment using anti-B19 immunoglobulin (IgG)-negative Japanese plasma under the same conditions used by the manufacturers of Kenketsu Venoglobulin®-IH, on a smaller scale.

Parvovirus B19-positive plasma

Anti-B19 IgG-negative, B19 DNA-positive plasmas that had been legally collected in the US were used. Plasma samples from three different donors, who were negative for human immunodeficiency virus, hepatitis B virus and hepatitis C virus (hereinafter referred to as P2, P3 and P4, respectively), were used (Yunoki *et al*, 2003).

Anti-B19 IgG antibody assay

The antibody titre of heat/PEG-treated IVIG (seven and three lots made of plasma procured in Japan and the US, respectively) was determined using parvovirus B19 IgG enzyme immunoassay (Biotrin International, Dublin, Ireland) and the World Health Organization International Reference (Anti-Parvovirus B19 Serum (IgG), Human, National Institute for Biological Standards and Control Catalogue No. 93/724).

Virus removal by 35 nm filter

The sample obtained by mixing B19-positive plasma and anti-B19 IgG (at 5°C, neutral pH, overnight), and the sample containing B19-positive plasma alone were resuspended (1/100 v/v) in 5% sorbitol solution containing heat/PEG-treated IVIG collected immediately before the removal step and the suspensions thus obtained were immediately subjected to filtration using a 35-nm Planova filter (Asahi Kasei Pharma, Tokyo, Japan). The quantity of B19 DNA before and after filtration was measured by the same method used previously (Yunoki *et al*, 2003).

Neutralization assay

Heat/PEG-treated IVIG was diluted threefold with 5% sorbitol and gradually diluted to an 81-fold dilution. B19-positive plasma (P4) was subjected to ultracentrifugation (150 000 *g* for 3 h). Plasma B19 was collected from the precipitate and resuspended in 5% sorbitol to adjust the number of two different virus samples (10·7 and 9·7 log copies/ml). The diluted heat/PEG-treated IVIG samples and the virus samples were mixed and left to react for 1 h at 37°C, and then assayed for any remaining infectivity of B19. Infectivity was determined by polymerase chain reaction detection of mRNA as an indicator, as previously reported (Yunoki *et al*, 2003).

Heat inactivation in liquid

Three different B19 samples derived from B19 positive plasmas (P2, P3 and P4) were ultracentrifuged as described above and added to the solution of heat/PEG-treated IVIG collected immediately before heat treatment. These three different mixtures were liquid-heat treated at 60°C for 2 h. B19 was added to the stabilizer alone to determine the effect of the stabilizer used in liquid heating (33% sorbitol). As in the previous report (Yunoki *et al*, 2003), measurement of infectivity in these studies used the detection of mRNA as an indicator.

As a control, we also evaluated the infectivity kinetics using CPV (1/10 v/v), followed by liquid-heat treatment at 60°C for 10 h. Infectivity in this study was assayed according to Yunoki *et al* (2003) with the appearance of cytopathic effects as an indicator.

Results

The anti-B19 IgG antibody titre in heat/PEG-treated IVIG by parvovirus B19 IgG enzyme immunoassay was (mean ± standard deviation, SD) 155·3 ± 5·7 IU/ml in those derived from Japanese plasmas collected in Japan and 255·8 ± 29·2 IU/ml in those from the US plasmas. In other words, anti-B19 IgG was present in all the lots tested and distribution of antibody titre was

Table I. Neutralization of parvovirus B19 heat/polyethylene glycol (PEG)-treated intravenous immunoglobulin (IVIG).

Dilution (fold)	Total IgG/ reaction (μg)	Total antibody titre (IU)	B19 DNA (9·7 log copies)	B19 DNA (8·7 log copies)
1	4500	14·97	–	–
3	1500	4·99	–	–
9	500	1·66	–	–
27	170	0·55	+	–
81	60	0·18	+	–

+, Infectious to KU812 cells; –, non-infectious to KU812 cells.

Table II. Effect on filtration ability of 35 nm Planova filter by the difference in reaction method with antibody.

	Method 1	Method 2
Before filtration	13·3*	11·5*
After filtration	8·5*	10·9*
LRF	4·8	0·6

Method 1, B19-antibody complex was added to heat/PEG-treated IVIG.
Method 2, B19 was added to heat/PEG-treated IVIG.
LRF, log reduction factor.
*Log copies of B19 DNA.

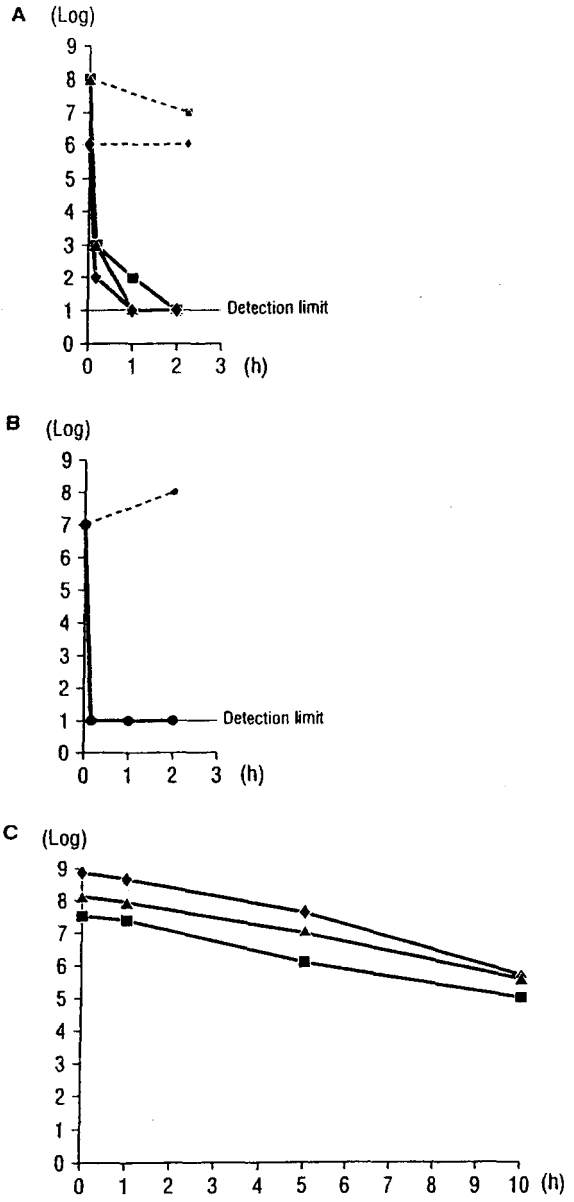

Fig 1. Viral inactivation kinetics in heat/polyethylene glycol (PEG)-treated intravenous immunoglobulin (IVIG) [B19 and canine parvovirus (CPV)] or stabilizer (B19): (A) Three different B19 samples derived from B19 DNA-positive plasmas P2 (◆), P3 (▲) and P4 (■) were added to the sample immediately before heat treatment of heat/PEG-treated IVIG, prepared from anti-B19 IgG-free plasma, and liquid-heat treated at 60°C for 2 h (solid line). In all cases, B19 was inactivated to below detection limits within 2 h of the treatment. The sample treated similarly at 37°C was used as a control (broken line). (B) B19 sample derived from B19 DNA-positive plasma was added to the stabilizer used in the heat treatment alone and then heat treated at 60°C (●, solid line). The sample treated similarly at 37°C was used as a control (broken line). When evaluated in stabilizer alone, B19 was inactivated within 15 min of the treatment to below detection limits. (C) Different infectious titres of CPV was added to the sample immediately before heat treatment of heat/PEG-treated IVIG, and liquid-heat treated at 60°C for 10 h (spike 1 (◆), spike 2 (▲) and spike 3 (■). In each case, CPV was slowly inactivated for 10 h.

not so wide. Although the data were obtained from only one lot, it was confirmed that heat/PEG-treated IVIG showed a neutralizing activity to inhibit B19 infection (Table I). In the study using the Planova filter with pore size of 35 nm, 4 logs or greater reduction of B19 virus was achieved when the sample was prepared by mixing antigen-positive plasma with antibody-positive plasma and leaving the mixture overnight. However, the B19 antigen-positive plasma sample showed no marked reduction. The result indicates that B19 may be too small to be removed by 35 nm Planova filtration if the virus exists by itself (Table II), suggesting that filtration of B19 with the Planova filter could have an effect on the conditions before the filtration.

The study using the sample collected in the process immediately before heat treatment, which was prepared from anti-B19 IgG-free plasma, showed that B19 was rapidly inactivated to below detection limits at 60°C (Fig 1A). This inactivation pattern was reproduced in all of the different B19-positive plasmas; therefore, we concluded that B19 was very easily inactivated, at least under these conditions. Similar rapid inactivation was observed in the heat treatment with the stabilizer (33% sorbitol) alone (Fig 1B). The study using CPV, the model virus of B19, showed that CPV was slowly inactivated with a considerably different pattern from B19 (Fig 1C).

Discussion

There have been several reports regarding B19 seroconversion after the administration of plasma derivatives. Blümel *et al* (2002b) closely investigated the genome similarity between the B19 obtained from the patients having received plasma fractionation preparations that had undergone vapour heat treatment (60°C for 10 h and 80°C for 1 h) or dry heat treatment (80°C for 72 h) and the B19 obtained from these preparations. They concluded that these inactivation treatments were not complete in inactivation of B19 (Blümel *et al*, 2002b). In addition, Hayakawa *et al* (2002) inferred that there could be a causal association between heat/PEG-treated IVIG subjected to liquid-heat treatment (60°C for 10 h) and B19 infection.

The heat treatment conditions used in these plasma derivatives differ from preparation to preparation and accordingly, the inactivation ability over B19 could differ greatly. Therefore, it is necessary to consider the ability to inactivate/remove B19 in each manufacturing process, when evaluating a causal association with B19 infection. However, as no technique using the infectivity of B19 as an indicator has been established to assess the risk of B19 infection that may be caused by plasma derivatives, virus inactivation ability of the heat treatment processes has been evaluated using model viruses such as CPV and PPV. Recently, we showed that B19 was heat sensitive under the liquid-heat treatment conditions employed in the manufacture of albumin preparation, although B19 was rather stable to the heat treatment conditions in 60% sucrose at 60°C for 1 h (Yunoki *et al*, 2003). In

the present report, we have evaluated the heat sensitivity of B19 under the liquid-heat treatment conditions used in the commercial production of heat/PEG-treated IVIG. As yet, it is not clear why 60% sucrose can protect B19 infectivity, at least for 1 h heating at 60°C.

Recently, Yokoyama *et al* (2004) reported that B19 formed aggregations in a solution containing amino acids such as glycine and therefore the aggregation could be removed by 35 nm filter. We are currently investigating how B19 and antibody, as well as B19 and sorbitol bind to each other.

The results of the clearance studies using multiple model viruses suggested the robustness of the manufacturing processes of heat/PEG-treated IVIG by inactivation and/or removal of viruses (data not shown). Further, this study confirmed that the liquid-heating step (60°C for 10 h) rapidly inactivated B19. Based on these findings, we propose that heat/PEG-treated IVIG could be safe for B19 for the following reasons: (i) robustness against virus contamination offered by its manufacturing process demonstrated in virus clearance studies; (ii) B19 inactivation process at 60°C for 10 h; and (iii) presence of neutralization antibodies in the final preparation.

Finally, we have not yet ascertained why one patient developed an infection after the administration of an immunoglobulin preparation that had undergone virus inactivation/removal treatment and contained anti-B19 IgG. One explanation at this point in time would be the possibility that the patient might have a history of previous B19 infection and the treatment, including administration of immunoglobulin, might have triggered reactivation of the B19 or antibody-dependent enhancement, as found in other viruses (Bloom *et al*, 2001; Takeda *et al*, 2003), although we do not know whether such latter possibilities can also occur *in vivo*.

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