TABLE 1. B19 DNA prevalence and levels in FVIII concentrates made during 1993 to 1998*

Product	VI/R and purification	Positive lots/lots tested (%)	B19 DNA (geq/mL) among positive lots†						
			10^6	$\geq 10^5$	$\geq 10^4$	$\geq 10^3$	$\geq 10^2$	≥ 10	Log geomean ± SEM
A	S/D, affinity, dry/80°C/72 hr	9/13 (69)	0	0	2	2	2	3	2.3 ± 0.41
B	S/D, immunoaffinity (SP)	22/25 (88)‡	0	0	7	10	3	2	3.0 ± 0.20§
C	S/D, immunoaffinity (RP)	14/15 (93)‡	0	2	2	8	2	0	3.3 ± 0.24§
D	S/D, gel filtration chromatography	15/15 (100)‡	2	6	1	2	3	1	3.9 ± 0.42§
E	Wet/60°C/10 hr	16/25 (64)	2	2	2	1	5	4	2.9 ± 0.45§
F	Wet/60°C/10 hr, immunoaffinity	24/43 (56)	0	0	0	3	11	10	1.7 ± 0.14
Total		100/136 (74)	4	10	14	26	26	20	

* Product C from recovered plasma (RP) and all other products from source plasma (SP); information regarding viral inactivation/removal (VI/R) and product purification procedures are from products' package inserts.

† B19 DNA–positive lots were categorized into groups, according to the level of B19 DNA. For 1993 to 1998, no positive lot was found to have fewer than 10 geq per mL; hence this category was not used. Categorization was done according to the following example: the at least 10^2 group indicates the number of positive lots containing less than 10^3 but at least 10^2 geq per mL. Levels in the last column are expressed as log geomean ± standard error of log geomean (SEM) among positive lots.

‡ $p < 0.01$ when compared to Product F; $p < 0.05$ when compared to Product E.

§ $p < 0.01$ when Product B, C, or D compared to Product F; $p < 0.05$ when Product E compared to Product F.

ing process are indicated in Table 1. Detectable B19 DNA in these products ranged from 56 to 100 percent of the lots tested (Table 1). The prevalence in either Product F or Product E was significantly lower than that in Products B, C, and D. Product F was a high-purity AHF product subjected to both wet heating at 60°C for 10 hours and purification by immunoaffinity chromatography utilizing monoclonal antibody (MoAb) to von Willebrand Factor (VWF), whereas Product E was an intermediate-purity product subjected only to wet heating. Interestingly, both Product B and Product C, which were S/D-treated, high-purity AHF products subjected to purification by immunoaffinity chromatography utilizing MoAb to FVIII, had a significantly higher positive rate than the wet-heated, high-purity Product F. In addition, the prevalences in lots of Products B and C, which were derived from source plasma and recovered plasma, respectively, were 88 and 93 percent, and hence there appeared to be no difference in prevalence of B19 as a function of the type of starting plasma. Three of the products, viz., A, E, and F, underwent manufacturing that included a heating step. When these were compared with the other products (B, C, and D), the prevalence was found to be significantly lower ($p < 0.001$), suggesting that heating was partially effective in eliminating detectable B19.

The highest level of B19 DNA in AHF products was 10^6 geq per mL found in 2 lots each of Products D and E (Table 1). Among 100 B19 DNA–positive lots, 54 lots (54% of the positive lots or 40% of the lots assayed) contained 10^3 geq per mL. Product F, which had the lowest prevalence of positive lots, also had the lowest B19 DNA levels among all products. The S/D-treated, immunoaffinity-purified Product B or Product C had significantly higher levels of B19 DNA when compared to Product F. There was no apparent difference in levels between Products B and C, which were derived from different types of starting plasma. Comparing the level of B19 DNA in heated product (A, E, and F) with that in unheated products (B, C, and D), suggested that heating was somewhat effective in lowering B19 contamination. Products E and F, however, differed significantly in B19 content ($p < 0.01$), despite the fact that both were heated for 10 hours at 60°C in solution during manufacture. Thus individual manufacturing steps (e.g., heating and immunoaffinity purification) can apparently be additive in their effects on B19.

Prevalence and levels of B19 DNA in lots manufactured during 2001 to 2004: effects of B19 NAT screening

To investigate the effect of minipool NAT screening by B19 NAT, we assayed AHF products made during 2001 to 2004 and compared the results with those from corresponding product lots made during 1993 to 1998. This appeared to be a valid comparison, inasmuch as the VI/R and product purification procedures for AHF products were essentially unchanged over this time span. The prevalence of B19 DNA in lots of products made from source plasma (i.e., all except Product C) ranged from 13 to 27 percent (Table 2). Moreover, all positive lots (25/129) made from source plasma contained less than 10^3 geq per mL, with 21 lots containing less than 10^2 geq per mL. Among 4 positive lots detected in Products A, B, and D, which had less than 10^3 but at least 10^2 geq per mL of B19 DNA, 3 lots were made in 2002, whereas 1 lot was made in 2004. In contrast, the prevalence in Product C, which was made from recovered plasma and had not been screened for B19 DNA, was 90 percent and the level of B19 DNA ranged as high as 10^4 geq per mL, with 9 of the 17 positive lots (53%) containing 10^3 geq per mL. Thus B19 NAT screening of plasma effectively lowered the B19 DNA level in all five products derived from source plasma, and in 81 percent of these 129 lots B19 DNA was undetectable. The product made

TABLE 2. B19 DNA prevalence and levels in FVIII concentrates made during 2001 to 2004

Product	Positive lots/lots tested (%)	B19 DNA (geq/mL) among positive lots*					
		$\geq 10^4$	$\geq 10^3$	$\geq 10^2$	≥ 10	<10	Log geomean ± SEM
A	8/30 (27)	0	0	2	1	5	1.8 ± 0.54
B	3/24 (13)	0	0	1	2	0	1.6 ± 0.43
C†	17/19 (90)‡	2	7	5	2	1	2.9 ± 0.25§
D¶	4/16 (25)	0	0	1	1	2	1.4 ± 0.55
E	6/28 (21)	0	0	0	3	3	0.86 ± 0.14
F	4/31 (13)	0	0	0	0	4	0.54 ± 0.04
Total (C excluded)	25/129 (19)	0	0	4	7	14	

* See Table 1.
† See Table 1.
‡ $p<0.01$ when compared to Product B.
§ $p<0.01$ when compared to Product B.
¶ Product subjected to additional dry heating at 80°C for 72 hours during this period.

TABLE 3. Anti-B19 IgG in FVIII concentrates*

Product	Positive lots/lots tested	
	1993-1998	2001-2004
A	0/4	0/5
B	1/11	0/5
C	0/9	0/4
D	0/3	0/4
E	10/10	8/8
F	0/13	0/4

* For Products B and C, lots (made in 2001-2004) tested for anti-B19 were not exclusively B19 DNA–positive.

from unscreened recovered plasma exhibited not only a prevalence that was virtually unchanged over the two time periods but also levels of B19 DNA that were not significantly decreased.

Anti-B19 IgG in FVIII concentrates

A small sampling of lots (most of which were B19 DNA–positive) from each product was analyzed for the presence of anti-B19 IgG. B19 IgG was detected in all lots of Product E, an intermediate-purity AHF product whose manufacturing involved no chromatographic or affinity purification step (Table 3). In contrast, all other products except 1 lot from Product B were found negative.

DISCUSSION

During the mid and late 1980s, manufacturing procedures for FVIII concentrates changed greatly because of the inclusion of steps designed to improve product purity and/or to achieve VI/R. Since the early 1990s, however, the product purification and VI/R procedures for AHF products have remained largely unchanged. In this study, the prevalence and levels of B19 DNA in 136 lots representing six AHF products, that is, three high-purity products (Products B, C, and F) and three intermediate-purity products (Products A, D, and E), made in 1993 to 1998, were evaluated. Products B and C were made by an identical manufacturing procedure, whereas all other products were made by different methods.

B19 DNA has been reported to be prevalent in AHF products with levels as high as 10^7 geq per mL.[6,8] In our study, it was also found in the majority (74%) of the AHF lots made during 1993 to 1998, with levels of B19 DNA up to 10^6 geq per mL. Moreover, we found that the prevalence of B19 DNA was 100 percent in early AHF lots made in the 1970s (data not shown). The manufacture of these lots involved no VI/R steps, and B19 DNA levels sometimes reached 10^8 geq per mL. Our data are consistent with numerous reported B19 transmissions by FVIII concentrates subjected to either S/D or heat treatment or both.[9-15]

The wet-heated, high-purity Product F had the lowest prevalence and levels of B19 DNA among six products tested. Product E underwent a similar wet-heat treatment and also exhibited a low prevalence. It contained high levels of B19 DNA, however, apparently because its manufacturing lacks the immunoaffinity purification procedure used for Product F. An even more effective removal procedure for B19 might be developed by further exploiting this immunoaffinity-chromatography step (utilizing anti-VWF). Interestingly, for Product B or C, the immunoaffinity-chromatography step utilizing anti-FVIII has been validated and found to remove 4 logs of a model virus for B19, yet the prevalence and levels remained relatively high compared to those of Product F.

Products A, E, and F, all of which underwent manufacturing that included a heating step, had a significantly lower prevalence and B19 DNA levels than did unheated Products B, C, and D, suggesting that heating was partially effective in eliminating detectable B19. Product A was subjected to S/D treatment and affinity purification plus dry heating in the final container at 80°C for 72 hours. B19 is known to withstand dry heating at high temperatures, however, and transmissions have been documented in recipients of such heated AHF products.[11,12] The possible role of affinity purification in removing and hence lower-

ing B19 DNA in Product A cannot be ruled out because this method is used in combination with the dry heating. In contrast, our data for wet-heated Products E and F are consistent with the recent findings[26,27] that B19 can be susceptible to inactivation when heated in certain liquid media.

We also attempted to evaluate the possible effect of the type of plasma used on the prevalence and level of B19 in the resulting AHF product. This came about because Products B and C were derived exclusively from source plasma and recovered plasma, respectively, but made by the same manufacturing procedure. To obtain a product lot of comparable size, more units of recovered plasma are needed when compared to source plasma because of the difference in the volume of an individual unit. The results, however, suggest that there was no apparent difference because a similar prevalence and level of B19 DNA in the resulting product were obtained.

The prevalence of B19 DNA in blood and plasma donors can vary widely,[2-4,28] probably reflecting whether the collections were done at the time of an epidemic. The degree of B19 viremia in positive donations can also span a wide range,[4,28] with lower B19 DNA levels usually found in anti-B19 IgG–positive donors.[28] Likewise, before the implementation of B19 NAT screening, levels of B19 DNA in manufacturing pools were reported to range widely,[3,4,6-8] reaching as high as 10^9 geq per mL. These variations can, possibly, give rise to fluctuations in the levels of B19 DNA in final products—such as those observed in this study. Nonetheless, the consistent statistical differences in prevalence and level of B19 (Table 1) led us to conclude that individual manufacturing steps may have a significant effect in clearing, that is, inactivating and/or removing, B19.

The introduction of B19 NAT screening of source plasma after 1999 afforded us an opportunity to evaluate the effect of the viral load in the starting plasma on both the prevalence and the level of B19 DNA in the final product. In AHF lots made in 1993 to 1998 (when B19 NAT screening was not yet implemented), a total of 40 percent of the 136 lots tested, or 54 percent of the B19 DNA–positive lots, contained 10^3 geq per mL. In contrast, in AHF lots made in 2001 to 2004, B19 NAT screening of plasma effectively lowered B19 DNA levels in all five products derived from source plasma so that, of the 129 lots tested, *none* had a level of 10^3 geq per mL, and in 81 percent of the lots tested B19 DNA was undetectable. Obviously B19 NAT screening had not yet been implemented during this period for the recovered plasma destined for Product C, since the results were similar to those obtained from unscreened plasma in the earlier period. That is, 47 percent of the 19 lots tested, or 53 percent of the 17 positive lots, contained 10^3 geq per mL of B19 DNA.

Detection of B19 DNA in the product does not necessarily equate with infectivity. The actual infectious level of B19 in products is likely to depend on the level of anti-B19 IgG that is copresent in the product, in addition to the recipient's immune status. Anti-B19 IgG have been considered to be neutralizing antibodies and appear to confer lasting protection.[1] Although low viremic levels were found in donors who seroconverted to anti-B19 IgG,[28] seropositive sera have been shown by an in vitro infectivity assay system to contain neutralizing antibodies to B19.[29] Other studies[2,30] involving the infusion of B19 DNA–positive blood products with B19 DNA up to 2×10^6 geq per mL also demonstrated that when anti-B19 IgG was present either in the recipients or in the products, there was no B19 infection. In a recent publication,[31] it was reported that two seropositive immunocompetent recipients with anti-B19 IgG levels of 19 and 39 IU per mL were not infected with B19 after receiving pooled plasma, S/D-treated containing high-titer B19 DNA (1.6×10^8 IU/mL) and anti-B19 IgG (72 IU/mL), whereas the seronegative recipients were infected. These studies have strongly suggested the protective role of anti-B19 IgG.

In our study, none of the products tested, with the exception of the intermediate-purity Product E and one lot of Product B, had detectable anti-B19 IgG. In view of the fact that at least 50 percent of adults have circulating anti-B19 IgG,[32,33] B19 antibodies will invariably be present in any large plasma pool. Interestingly, most manufacturing procedures for AHF must be effective in separating FVIII from IgG. A product containing B19 DNA but devoid of anti-B19 IgG would potentially be infectious in seronegative recipients.

The minimal infectious dose (in terms of B19 DNA) in seronegative individuals is unknown. In a recent case report, we found that infection occurred when a seronegative, immunocompetent patient with mild hemophilia received an S/D-treated, high-purity AHF product, which contained 10^3 geq per mL of B19 DNA and was devoid of any detectable anti-B19 IgG; the total dose infused was 2×10^4 geq.[15] This is the lowest infectious dose (in terms of B19 DNA) that has been reported for a product containing no anti-B19 IgG. In a separate transmission case, a seronegative child was also infected by infusing a dry-heat-treated FVIII concentrate, which contained 4×10^3 geq per mL B19 DNA, but the total dose in terms of B19 DNA was 4×10^6 geq, and the product's anti-B19 IgG content, if any, was unknown.[14] For pooled plasma, S/D-treated, which was anti-B19 IgG–positive, a higher dose of B19 DNA was needed to produce infection. Only those seronegative volunteers infused with a 200-mL dose of product lots containing more than 10^7 geq per mL B19 DNA were infected, whereas those infused with an equal volume of lots containing less than 10^4 geq per mL did not seroconvert.[16,17,19] It remains to be determined whether AHF products containing low residual levels of B19 still may transmit the infection to susceptible individuals (presumably seronegative persons), especially to those high-risk individuals who are immune deficient.

B19 virus isolates have recently been classified into three genotypes with the majority of the isolates grouped as Genotype 1.[34] Like Genotype 1, Genotype 2 DNA was detected in FVIII concentrates as a contaminant but with lesser prevalence (2.5%); interestingly all Genotype 2-positive lots were also Genotype 1-positive.[35] The infectivity of a B19 Genotype 2 virus was recently found to be similar to that of Genotype 1 in an in vitro assay.[36] Genotype 3 has not been reported in FVIII concentrates. The lack of such a report, however, may be due to the fact that some B19 NAT procedures detect only Genotype 1 but not variant Genotype 2 or 3.[37] The NAT procedure used in this study detects both Genotype 1 and Genotype 2 but does not detect Genotype 3.

In conclusion, we have demonstrated that the prevalence and levels of B19 in FVIII concentrates made from plasma that was not screened for B19 DNA were high but varied among products with different manufacturing procedures. Minipool NAT screening for B19 DNA effectively lowers the prevalence and level of B19 in AHF. The majority of the lots of AHF now being manufactured have no detectable B19 DNA, and thus the risk of B19 transmission may be greatly reduced.

ACKNOWLEDGMENTS

We thank J.S. Finlayson, PhD, for critical review of the manuscript. We acknowledge AHF manufacturers for providing some samples and the lot-associated information when needed.

REFERENCES

1. Young NS, Brown KE. Mechanisms of disease: parvovirus B19. N Engl J Med 2004;350:586-97.
2. Jordan J, Tiangco B, Kiss J, Koch W. Human parvovirus B19: prevalence of viral DNA in volunteer blood donors and clinical outcomes of transfusion recipients. Vox Sang 1998;75:97-102.
3. Aubin JT, Defer C, Vidaud M, et al. Large-scale screening for human parvovirus B19 DNA by PCR: application to the quality control of plasma for fractionation. Vox Sang 2000; 78:7-12.
4. Weimer T, Streichert S, Watson C, Gröner A. High-titer screening PCR: a successful strategy for reducing the parvovirus B19 load in plasma pools for fractionation. Transfusion 2001;41:1500-4.
5. Siegl G, Cassinotti P. Presence and significance of parvovirus B19 in blood and blood products. Biologicals 1998;26: 89-94.
6. Saldanha J, Minor P. Detection of human parvovirus B19 DNA in plasma pools and blood products derived from these pools: implications for efficiency and consistency of removal of B19 DNA during manufacture. Br J Haematol 1996;93:714-9.
7. Eis-Hübinger AM, Sasowski U, Brackmann HH. Parvovirus B19 DNA contamination in coagulation factor VIII products. Thromb Haemost 1999;81:476-7.
8. Schmidt I, Blümel J, Seitz H, et al. Parvovirus B19 DNA in plasma pools and plasma derivatives. Vox Sang 2001;81: 228-35.
9. Azzi A, Ciappi S, Zakvrzewska K, et al. Human parvovirus B19 infection in hemophiliacs first infused with two high-purity, virally attenuated factor VIII concentrates. Am J Hematol 1992;39:228-30.
10. Azzi A, Morfini M, Mannucci PM. The transfusion-associated transmission of parvovirus B19. Transfus Med Rev 1999;13:194-204.
11. Yee TT, Cohen BJ, Pasi KJ, Lee CA. Transmission of symptomatic parvovirus B19 infection by clotting factor concentrate. Br J Haematol 1996;93:457-9.
12. Santagostino E, Mannucci PM, Gringeri A, et al. Transmission of parvovirus B19 by coagulation factor concentrates exposed to 100 degrees C heat after lyophilization. Transfusion 1997;37:517-22.
13. Matsui H, Sugimoto M, Tsuji S, et al. Transient hypoplastic anemia caused by primary human parvovirus B19 infection in a previously untreated patient with hemophilia transfused with a plasma-derived, monoclonal antibody-purified factor VIII concentrate [case report]. J Pediatr Hematol Oncol 1999;21:74-6.
14. Blümel J, Schmidt I, Effenberger W, et al. Parvovirus B19 transmission by heat-treated clotting factor concentrates. Transfusion 2002;42:473-81.
15. Wu CG, Mason B, Jong J, et al. Parvovirus B19 transmission by a high-purity factor VIII concentrate. Transfusion 2005; 45:1003-10.
16. Blood Products Advisory Committee. Nucleic acid testing of blood donors for human parvovirus B19 [transcript on the Internet]. Rockville (MD): U.S. Food and Drug Administration; 1999. Available from: http://www.fda.gov/ohrms/dockets/ac/99/transcpt/3548t1.rtf
17. Davenport R, Geohas G, Cohen S, et al. Phase IV study of PLAS+ SD: hepatitis A (HAV) and parvovirus B19 safety results. Blood 2000;96:451a.
18. Koenigbauer U, Eastlund T, Day JW. Clinical illness due to parvovirus B19 infection after infusion of solvent/detergent-treated pooled plasma. Transfusion 2000;40: 1203-6.
19. Brown KE, Young NS, Alving BM, Barbosa LH. Parvovirus B19: implications for transfusion medicine. Summary of a workshop. Transfusion 2001;41:130-5.
20. Tabor E, Yu MW, Hewlett I, Epstein JS. Summary of a workshop on the implementation of NAT to screen donors of blood and plasma for viruses. Transfusion 2000;40: 1273-5.
21. Hewlett IK, Yu MW, Epstein JS. Implementation of donor screening for infectious agents transmitted by blood by nucleic acid technology. The International Forum. Vox Sang 2002;82:87-111.

22. Tabor E, Epstein JS. NAT screening of blood and plasma donations: evolution of technology and regulatory policy. Transfusion 2002;42:1230-7.
23. Blood Products Advisory Committee. Human parvovirus B19 testing for whole blood and source plasma [transcript on the Internet]. Rockville (MD): U.S. Food and Drug Administration; 2002. Available from: http://www.fda.gov/ohrms/dockets/ac/02/transcripts/3913t1.rtf
24. Aberham C, Pendl C, Gross P, et al. A quantitative, internally controlled real-time PCR assay for the detection of parvovirus B19 DNA. J Virol Methods 2001;92:183-91.
25. Saldanha J, Lelie N, Yu MW, Heath A; B19 Collaborative Study Group. Establishment of the first World Health Organization International Standard for human parvovirus B19 DNA nucleic acid amplification techniques. Vox Sang 2002; 82:24-31.
26. Blümel J, Schmidt I, Willkommen H, Löwer J. Inactivation of parvovirus B19 during pasteurization of human serum albumin. Transfusion 2002;42:1011-8.
27. Yunoki M, Tsujikawa M, Urayama T, et al. Heat sensitivity of human parvovirus B19. Vox Sang 2003;84:164-9.
28. Thomas I, Di Giambattista M, Gérard C, et al. Prevalence of human erythrovirus B19 DNA in healthy Belgian blood donors and correlation with specific antibodies against structural and non-structural viral proteins. Vox Sang 2003; 84:300-7.
29. Wong S, Brown KE. Development of an improved method of detection of infectious parvovirus B19. J Clin Virol 2006; 35:407-13.
30. Plentz A, Hahn J, Knöll A, et al. Exposure of hematologic patients to parvovirus B19 as a contaminant of blood cell preparations and blood products. Transfusion 2005;45: 1811-5.
31. Doyle S, Corcoran A. The immune response to parvovirus B19 exposure in previously seronegative and seropositive individuals. J Infect Dis 2006;194:154-8.
32. Anderson LJ, Tsou C, Parker RA, et al. Detection of antibodies and antigens of human parvovirus B19 by enzyme-linked immunosorbent assay. J Clin Microbiol 1986;24: 522-6.
33. Cohen BJ, Buckley MM. The prevalence of antibody to human parvovirus B19 in England and Wales. J Med Microbiol 1988;25:151-3.
34. Servant A, Laperche S, Lallemand F, et al. Genetic diversity within human erythroviruses: identification of three genotypes. J Virol 2002;76:9124-34.
35. Schneider B, Becker M, Brackmann HH, Eis-Hübinger AM. Contamination of coagulation factor concentrates with human parvovirus B19 genotype 1 and 2. Thromb Haemost 2004;92:838-45.
36. Blümel J, Eis-Hübinger AM, Stühler A, et al. Characterization of parvovirus B19 genotype 2 in KU812Ep6 cells. J Virol 2005;79:14197-206.
37. Baylis SA, Shah N, Minor PD. Evaluation of different assays for the detection of parvovirus B19 DNA in human plasma. J Virol Methods 2004;121:7-16.

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			報告日	第一報入手日 2007 年 5 月 22 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	人ハプトグロビン		研究報告の公表状況	Journal of virology 2007; 81(8): 4130-4136	公表国 スウェーデン	
販売名 (企業名)	ハプトグロビン注－ヨシトミ(ベネシス)					

研究報告の概要

我々は先に、臨床サンプルについての大規模な分子的なウイルススクリーニングのシステムを報告した。まだ発見されていないヒトの病原体を組織的に探索する努力の一環として、この技術を、ヒトの気道からの検体をもとにしたウイルスのスクリーニングに適用した。その結果、以前には知られていなかった KI ポリオーマウイルスと暫定的に名付けたポリオーマウイルスを同定した。このウイルスは、遺伝子の early 領域では、他の霊長類のポリオーマウイルスに系統樹的に近似するが、late 領域では、既知のポリオーマウイルスに対して相同性が少ない（アミノ酸の 30%未満が同一）。このウイルスは、PCR によって、鼻咽頭吸引物の 6/637 (1%) と便検体の 1/192 (0.5%) に見出されたが、尿及び血液の検体には見出されなかった。ポリオーマウイルスは発癌の可能性を有し、免疫が抑制されたヒトに重篤な疾患を起こす可能性があることから、様々な医療状況下で、このウイルスを探索し続けることが重要である。

報告企業の意見	今後の対応
ヒトの気道からの検体から、以前には知られていなかった KI ポリオーマウイルスと暫定的に名付けたポリオーマウイルスを同定したとの報告である。なお、このウイルスと近似のウイルスの発見報告を BENE2007-013 で報告する。 ポリオーマウイルスは、直径 40nm のエンベーロープを有しない DNA ウイルスである。万一原料血漿にポリオーマウイルスが混入したとしても、EMC 及び CPV をモデルウイルスとしたウイルスバリデーション試験成績から、本剤の製造工程において十分に不活化・除去されると考えている。	本報告は本剤の安全性に影響を与えないと考えるので、特段の措置はとらない。

使用上の注意記載状況・その他参考事項等

2. 重要な基本的注意
(1) 本剤の原材料となる献血者の血液については、HBs 抗原、抗 HCV 抗体、抗 HIV-1 抗体、抗 HIV-2 抗体、抗 HTLV-Ⅰ抗体陰性で、かつ ALT(GPT)値でスクリーニングを実施している。更に、プールした試験血漿については、HIV-1、HBV 及び HCV について核酸増幅検査(NAT)を実施し、適合した血漿を本剤の製造に使用しているが、当該 NAT の検出限界以下のウイルスが混入している可能性が常に存在する。本剤は、以上の検査に適合した血漿を原料として、Cohn の低温エタノール分画で得た画分から人ハプトグロビンを濃縮・精製した製剤であり、ウイルス不活化・除去を目的として、製造工程において 60℃、10 時間の液状加熱処理及び濾過膜処理(ナノフィルトレーション)を施しているが、投与に際しては、次の点に十分注意すること。

JOURNAL OF VIROLOGY, Apr. 2007, p. 4130–4136 Vol. 81, No. 8
0022-538X/07/$08.00+0 doi:10.1128/JVI.00028-07

Identification of a Third Human Polyomavirus▽

Tobias Allander,[1]* Kalle Andreasson,[2] Shawon Gupta,[1] Annelie Bjerkner,[1] Gordana Bogdanovic,[1,2] Mats A. A. Persson,[3] Tina Dalianis,[2] Torbjörn Ramqvist,[2] and Björn Andersson[4]

Laboratory for Clinical Microbiology, Department of Microbiology, Tumor and Cell Biology, Karolinska University Hospital, Karolinska Institutet, SE-17176 Stockholm, Sweden[1]; Cancer Center Karolinska, Department of Oncology and Pathology, Karolinska University Hospital, Karolinska Institutet, SE-17176 Stockholm, Sweden[2]; Center for Molecular Medicine, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, SE-17176 Stockholm, Sweden[3]; and Department of Cell and Molecular Biology, Karolinska Institutet, SE-17177 Stockholm, Sweden[4]

Received 5 January 2007/Accepted 30 January 2007

We have previously reported on a system for large-scale molecular virus screening of clinical samples. As part of an effort to systematically search for unrecognized human pathogens, the technology was applied for virus screening of human respiratory tract samples. This resulted in the identification of a previously unknown polyomavirus provisionally named KI polyomavirus. The virus is phylogenetically related to other primate polyomaviruses in the early region of the genome but has very little homology (<30% amino acid identity) to known polyomaviruses in the late region. The virus was found by PCR in 6 (1%) of 637 nasopharyngeal aspirates and in 1 (0.5%) of 192 fecal samples but was not detected in sets of urine and blood samples. Since polyomaviruses have oncogenic potential and may produce severe disease in immunosuppressed individuals, continued searching for the virus in different medical contexts is important. This finding further illustrates how unbiased screening of respiratory tract samples can be used for the discovery of diverse virus types.

Persistent virus infections are an integrated part of human life. Most humans are persistently infected with one or more herpesviruses, papillomaviruses, polyomaviruses, and anelloviruses and remain healthy. Nevertheless, many of these viruses may occasionally produce severe disease (21, 32, 34, 37). Identification of previously unrecognized viral species is technically difficult. Thus, many potentially medically important persisting human viruses most likely remain undetected.

Polyomaviruses are small DNA viruses capable of persistent infection and having oncogenic potential. They have been found in many mammals and birds worldwide. Two polyomaviruses are known to normally infect humans, JC virus (JCV) and BK virus (BKV), both discovered in 1971 (13, 30). They are genetically closely related to each other, and both viruses show 70 to 80% seroprevalence in adults (23). The routes of acquisition and sites of primary infection are largely unknown, but both viruses can establish a latent infection in the kidneys and, in the case of JCV, also in the central nervous system (31). Persistent replication in the kidneys is evidenced by the fact that JCV, and occasionally also BKV, can be detected in the urine of healthy adults (23). BKV has also been detected in the feces of children (35). JCV and BKV are highly oncogenic in experimental animals, but a role in the development of human tumors has not been established (25). Disease caused by human polyomaviruses has been observed in immunosuppressed individuals. JCV is the causative agent of progressive multifocal leukoencephalopathy, a demyelinating disease of the brain and a feared complication of AIDS (21). This disorder has recently received renewed attention after the occurrence of fatal cases among patients treated with natalizumab for multiple sclerosis (22, 24). BKV has been associated with post-transplantation nephropathy and hemorrhagic cystitis in hematopoietic stem cell transplant (HSCT) recipients (7, 17). In addition to JCV and BKV, there are reports on the presence of the primate polyomavirus simian virus 40 (SV40) in humans, possibly introduced by contaminated poliovirus vaccine produced in monkey cells (4), although other ways of transmission have also been suggested (10, 27). SV40 genomic sequences have been detected in human malignant mesothelioma tumors, but its role in human tumor development remains debated (25).

We have developed a system for large-scale molecular screening of human diagnostic samples for unknown viruses (2). With this technology, we have initiated a systematic search for previously unrecognized viruses infecting humans in order to identify agents that are potentially involved in human disease. We describe here the identification and molecular characterization of a hitherto unknown human polyomavirus, which is only distantly related to the other known primate polyomaviruses. In analogy with the nomenclature of the other human polyomaviruses, we propose the name KI polyomavirus, KIPyV, for the newly discovered virus.

MATERIALS AND METHODS

Molecular virus screening. As part of a systematic search for unknown viruses in clinical respiratory tract samples, a screening library was constructed from cell-free supernatants of 20 randomly selected nasopharyngeal aspirates made anonymous and submitted to the Karolinska University Laboratory, Stockholm, Sweden, for the diagnosis of respiratory tract infections. The samples were collected from March to June of 2004 and stored at −80°C until analyzed. This study was approved by the Karolinska Institutet local ethics committee. The procedure used for identification of virus nucleic acid sequences, molecular virus screening, has been described previously (2). In brief, samples were pooled and the pool was divided into two aliquots, which were filtered through 0.22- and 0.45-μm-pore-size disc filters (Millex GV/HV; Millipore), respectively. Both aliquots were ultracentrifuged at 41,000 rpm in an SW41 rotor (Beckman) for 90

* Corresponding author. Mailing address: Department of Clinical Microbiology, L2:02, Karolinska University Hospital, SE-171 76 Stockholm, Sweden. Phone: 46 8 51773580. Fax: 46 8 308099. E-mail: tobias.allander@karolinska.se.

▽ Published ahead of print on 7 February 2007.