3F4 and 4F2 was used on the hypothesis that although MoAb 4F2 clearly identifies PrP on human RBCs whereas MoAb 3F4 does not bind to human RBCs,[28] the 4F2 epitope on any PrP^{Sc} may be more susceptible to pK cleavage than the 3F4 epitope. These MoAbs (5 µL each at 0.5 mg/mL) were added simultaneously to 100 µL of whole blood or pK-treated washed blood with 100 µL of cell wash. Second samples of whole blood and pK-treated blood received 100 µL of cell wash without anti-prion MoAbs as unstained (negative) control. After incubation and washing, anti-mouse immunoglobulin (human absorbed fluorescein isothiocyanate [FITC]-conjugated goat anti-mouse immunoglobulin $F(ab')_2$, Biosource, Camarillo, CA) at 1 in 500 in cell wash (100 µL) was added to all tubes. After incubation and washing, a sample (10 µL) was removed from each tube for RBC studies, and 5 µL of peridinin chlorophyll protein–conjugated anti-CD45 MoAb and 5 µL of phycoerythrin (PE)-conjugated anti-CD41 MoAb (Becton Dickinson) were added to each whole-blood tube. To each RBC study sample, 5 µL of PE-conjugated anti-glycophorin A MoAb (Serotec, Oxford, UK) was added. Samples were incubated an additional 30 minutes in the dark at room temperature. The RBC samples were washed twice with 2.5 mL of cell wash and resuspended in 0.5 mL of cell fix (Becton Dickinson). The whole-blood samples were resuspended in 2.5 mL of lysing solution (Becton Dickinson) to remove RBCs followed by two washes in 2.5 mL of cell wash and resuspended in 0.6 mL of cell fix. Fixed stained samples were kept overnight at 4°C in the dark before analyzing by three-color flow cytometry as previously described.[30] Samples from lysed preparations were collected on linear forward- and side-scatter axes for WBC studies, and a second set was collected on logarithmic forward- and side-scatter axes for PLT studies. RBCs were collected on linear forward- and side-scatter axes. A total of 20,000 events in appropriate forward- and side-scatter gates were collected to listmode files for each analysis. Analysis was conducted with computer software (FCS Express, DeNovoSoftware, Thornhill, Ontario, Canada) on a personal computer. Samples were gated by appropriate forward- and side-scatter patterns and additionally by CD45 expression (different WBC populations), CD41 expression (PLTs), or glycophorin A expression (RBCs). Other markers of WBC populations (e.g., CD14) were susceptible to pK digestion and were not used. Histograms were made of channel-1 (FITC) expression for negative controls (no anti-PrP MoAbs) and overlaid with histograms for channel-1 for anti-PrP (primary anti-PrP MoAbs) for each cell class: single peaks were observed in each case and their median fluorescence intensities determined by setting appropriate markers on the histograms. Net median fluorescence intensity for anti-PrP staining was obtained by subtracting the negative control (no anti-PrP MoAbs) value.[30]

Statistical analysis

Clinical groups were compared with the U test carried out with computer software (NCSS 2001 software, NCSS, Kaysville, UT). Box plots were produced by the NCSS 2001 software according to a common procedure in which boxes represent the interquartile range (IQR), the top and bottom of the box are the 25th and 75th percentiles, and the horizontal line through the box represents the median. The line and bar (whiskers) above and below the box represent the upper and lower adjacent values. The upper adjacent value is the largest observation that is not greater than the 75th percentile plus 1.5 times the IQR. The lower adjacent value is the smallest observation that is at least the 25th percentile minus 1.5 times the IQR. Outliers, shown as small circles, are those values that lie outside the upper and lower adjacent values.[31]

RESULTS

Detection of whole-blood PrP^c by DELFIA

Whole-blood samples from 10 patients with vCJD, 10 patients with sCJD, 8 neurological controls, and 29 healthy adults (1 excluded owing to lack of parallelism between dilutions) were analyzed in duplicate at five dilutions by DELFIA. For each group the median and IQR were calculated (Fig. 1A; Table 1). There is a significant decrease in the concentration of PrP^c in vCJD ($p = 0.012$) and neurological control patients ($p = 0.0004$) compared with healthy adults, but not between vCJD and sCJD, nor between neurological controls and sCJD patients. Despite the significance, there was considerable overlap between the vCJD and healthy adult control groups, which indicates that this observation would have little use as a discriminatory test for diagnosis or screening. These differences between groups were retained and continued to be significant when PrP^c concentration is not normalized for total protein but is expressed directly as units per milliliter; there remains a significant decrease in the concentration of PrP^c in vCJD ($p = 0.005$) and neurological control patients ($p = 0.0001$) when compared with healthy adults.

Detection of PLT-poor plasma PrP^c by DELFIA

PLT-poor plasma samples from 10 patients with vCJD, 10 patients with sCJD, 29 healthy adults (1 excluded owing to lack of parallelism between dilutions), and 6 samples (2 samples unavailable) from neurological controls were analyzed by DELFIA for PrP^c (Fig. 1B; Table 1). We found significant elevation in the plasma PrP^c concentration in sCJD patients when compared with both healthy adult ($p = 0.022$) and neurological control groups ($p = 0.050$), but not when compared to levels found in vCJD patients. No significant differences were found in comparisons

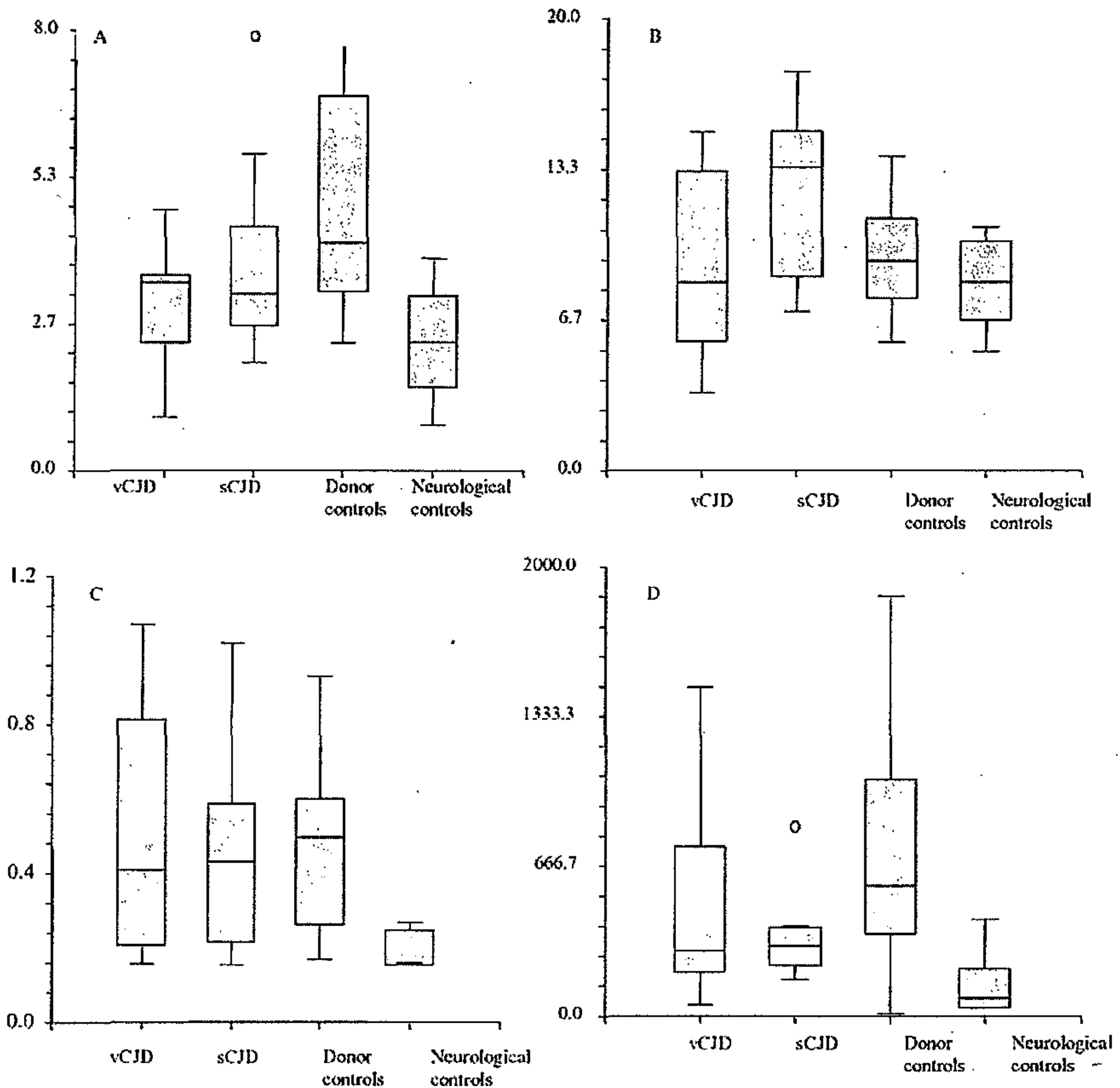

Fig. 1. The concentration of PrP^c assayed by DELFIA in separated blood components of clinical and control samples. The concentration of whole blood (A), PLT-poor plasma (B), 50 percent RBCs (C), and PLT (D) PrP^c (units per mg) assayed by DELFIA in vCJD, sCJD, and donor and neurological control groups.

between controls and vCJD groups. Again, there is considerable overlap of ranges for all groups. Significant differences between groups were not evident when data was expressed in units per milliliter of PrP^c.

Detection of RBC PrP^c by DELFIA

RBC samples from 10 vCJD patients, 9 sCJD patients, 26 healthy adults, and 7 neurological controls were analyzed by DELFIA to determine PrP^c concentration (Fig. 1C; Table 1). vCJD and sCJD patients and healthy adults all exhibited similar median values for RBC PrP^c concentration and therefore did not exhibit significant differences when compared with each other. The neurological control group showed a significant reduction in RBC PrP^c concentration when compared with vCJD patients ($p = 0.029$), sCJD patients ($p = 0.024$), and healthy adults ($p = 0.001$).

TABLE 1. Medians and range (95% confidence limits [CL]; U/mg) of separated components from different clinical and control groups as measured by DELFIA

Sample	Blood component	Number	Median (U/mg)	95% CL (U/mg)
vCJD	Whole blood	10	3.438	1.912-3.593
sCJD	Whole blood	10	3.227	2.073-5.754
Donor controls	Whole blood	29	4.16	3.376-6.635
Neurological controls	Whole blood	8	2.35	0.823-3.375
vCJD	Plasma	10	8.375	5.225-14.912
sCJD	Plasma	10	13.446	8.078-17.314
Donor controls	Plasma	29	9.316	8.038-10.624
Neurological controls	Plasma	6	8.405	5.31-10.8
vCJD	RBCs	10	0.411	0.19-1.045
sCJD	RBCs	9	0.434	0.162-0.648
Donor controls	RBCs	26	0.499	0.29-0.579
Neurological controls	RBCs	7	0.16	0.16-0.27
vCJD	PLTs	9	293.93	174.42-1150.442
sCJD	PLTs	9	316.56	213.69-400.75
Donor controls	PLTs	24	581.11	389.29-855.15
Neurological controls	PLTs	7	83.37	35.48-429.6

Detection of PLT PrP^c by DELFIA

PLT samples from 24 healthy adults, 9 patients with vCJD, 9 patients with sCJD, and 7 neurological controls were analyzed for PrP^c concentration (Fig. 1D; Table 1). Samples excluded from each group constitute those where PrP^c concentration could not be measured reproducibly across a range of dilutions. The concentration of PLT PrP^c in the sCJD samples was significantly reduced compared with levels in healthy adults ($p = 0.021$) but not against vCJD. The level of PLT PrP^c in neurological controls was the lowest of all groups, and this reduction was significant compared to levels in healthy adults ($p = 0.001$), vCJD patients ($p = 0.039$), and sCJD patient samples ($p = 0.017$).

Detection of buffy-coat PrP^c by DELFIA

Buffy-coat PrP^c expression levels were not detected at significantly high concentrations, particularly in clinical groups, to permit accurate measurement. Hence, they were excluded from the analysis.

Flow cytometry

The cell-associated PrP (net median fluorescence intensity above background) ranges for lymphocytes, PLTs, and RBCs are shown as interquartile box plots (Fig. 2) for healthy adult controls, neurological controls, sCJD cases, and vCJD cases. The only significant difference found between groups was for RBC PrP in healthy adults compared to non-CJD neurological controls ($p = 0.008$). Although PLT PrP was increased in both sCJD and vCJD cases compared to healthy adults or neurological controls, this did not reach significance. Clinical vCJD and sCJD cases were found to be as sensitive as healthy adults and non-CJD neurological patients to removal of cell-associated PrP by pK (Fig. 3). The distribution of FITC fluorescence after pK treatment was homogeneous, showing a single low peak in the FITC channel without any discernible higher peak, which might indicate the retention of protease K–resistant PrP, putative PrP^{Sc}, on any subpopulation of cells.

Results are not reported for neutrophils or monocytes because in these studies we considered the results unreliable. There appeared to be considerable nonspecific binding of fluorescence by both WBC classes especially evident in both healthy adult controls and all clinical cases, which may have been a consequence of sample age.

Stability of whole-blood PrP^c

Some samples from clinical groups took longer than 24 hours to arrive in the laboratory. To consider any effects of prolonged transit time at ambient temperature, whole-blood samples from three healthy adult controls were left at 22°C for 72 hours, and samples were removed from each control at 24-hour intervals. Samples were analyzed at each time by flow cytometry, and 1-mL samples for DELFIA analysis were stored at −80°C until analysis for the detection of whole-blood PrP^c. The expression of PrP on PLTs measured by flow cytometry at 24-hour intervals showed sequentially decreasing levels of PrP with time for each individual (not shown). This contrasted with identification of increased expression of PrP on PLTs found in a preliminary analysis of data for sCJD patients compared with healthy adult controls, and implied sample storage was not responsible for this increase in the patient group. Levels of PrP^c detected by DELFIA remained stable across 72 hour (not shown). Prolonged transit time and ambient storage temperatures had negligible effects on PrP^c concentration and are therefore unlikely to contribute to differences between sample groups.

Relationship between age and PrP^c levels

Differences between control and clinical sample groups may be affected by the age of individuals, considering that vCJD usually affects young people and sCJD older people. The concentration of PrP^c (U/mg) in whole-blood samples detected by DELFIA in CJD and control groups was plotted against age and showed that PrP^c expression is unrelated to this variable (data not shown).

Hematology of clinical and control samples

To ensure that hematologic abnormalities in blood samples from clinical patients and controls did not contribute

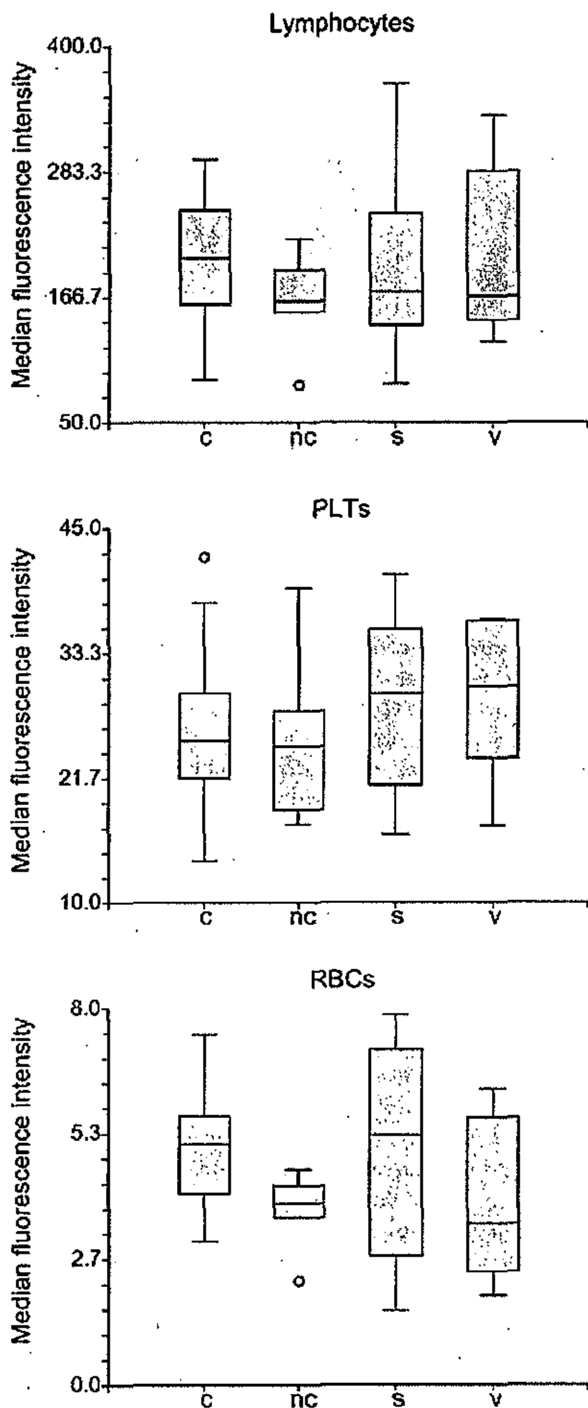

Fig. 2. Box plots showing the ranges of expression of cellular PrP shown by flow cytometry on different clinical and control groups. Expressed as median fluorescence intensity net of background, on lymphocytes, RBCs, and PLTs.

to differences in PrP concentration and expression between groups, full blood count data for clinical and neurological patients were reviewed. No gross abnormalities were detected (data not shown).

DISCUSSION

The DELFIA technique employed here, and described previously by us,[23] is well suited to the screening of whole blood and separated components of clinical and control samples for PrP^c. The assay is quick and has a high sensitivity for the detection of PrP^c. DELFIA technology is compatible with high throughput and has a high signal to background ratio, owing to background deteriorating rapidly whereas the signal has a long lifetime. Flow cytometry analysis has been used previously by us to illustrate the expression of PrP on healthy adult human blood cells.[29,30]

No previous studies have evaluated the concentration and expression of PrP in the whole blood and separated components of patients with vCJD. DELFIA analysis of separated components found that the highest levels of PrP^c associated with the PLT and plasma components and much lower levels associated with RBCs, which is in agreement with our previously described findings.[23] Levels of PrP^c in the WBC buffy-coat fractions were very low, often at the detection limit of the assay, and these levels could not be accurately calculated particularly in clinical groups, hence their exclusion from further analysis. The separation method used here was a compromise for greatest utility of archived samples and was not ideal for isolation of the PLT and buffy-coat components; cells often clumped together and proved difficult to resuspend, which may explain the large median ranges in the PLT data and the low levels of detectable PrP^c in buffy coats. These large ranges in PLT PrP^c concentrations are not supported by any large fluctuations in PLT counts as seen in hematologic data for patients and controls used in this study; the data does not show any abnormalities.

In our analysis of whole blood, we found a significant decrease in the concentration of PrP^c in vCJD and neurological control patient samples compared to healthy adults but not when compared with the sCJD group. We found significant elevation in the plasma PrP^c concentration in sCJD patients when compared with both healthy adult and neurological control groups; however, no significant differences were evident between levels in sCJD and vCJD patients. The elevation of plasma PrP^c in sCJD patients agrees with a previously published report; however, in our study a distinct finding is that levels of plasma PrP^c in sCJD patients are significantly elevated not only against healthy adults but also against the neurological control group, which was not the case in the previous published findings.[25] These differences may be a consequence of our use of neurological controls with conditions, apart from Alzheimer's disease, that were distinct from those

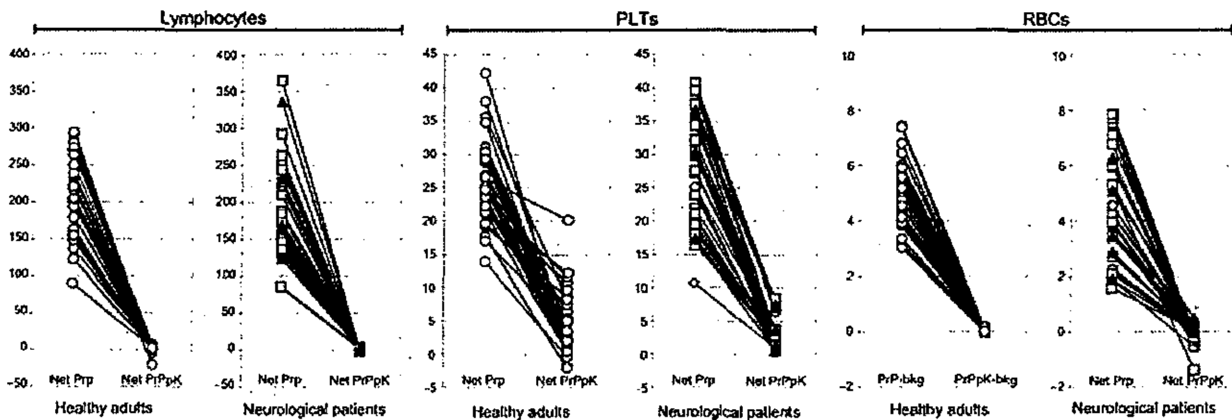

Fig. 3. Demonstration by flow cytometry of the removal of cell-surface PrP after pK treatment. The median fluorescence intensity (net of background) is plotted for cells before and after pK treatment and connected by a line for each case. (○) Healthy adult blood donors; (○) non-CJD neurological patients; (□) sCJD patients; (▲) vCJD patient; (◇) familial TSE (a single case of familial TSE was included in the study but not included in statistical analysis).

included in the previous study. We found a significant reduction in the concentration of RBC PrP^c in neurological controls compared with all other groups. We considered that the differences between groups might be a consequence of the age of patients affected by vCJD, sCJD, and neurological disorders; however, no relationship between age and PrP^c concentration was found in either control or clinical sample groups. It is possible that the reduced concentration of whole-blood PrP^c in vCJD patient samples might be due to the conversion of PrP^c to PrP^{Sc} as part of the replication process involved in disease pathogenesis. The absence of a similar reduction of whole-blood PrP^c in sCJD patients would be expected because PrP^{Sc} is largely confined to the central nervous system and replication of PrP^{Sc} in the periphery before neuroinvasion is not a feature of sCJD. Alternatively, the vCJD whole-blood reduction could be a summation of reductions in PrP^c in the plasma and PLT components, whereas in sCJD the low PLT PrP^c value is offset by the high plasma value so balancing out in whole-blood levels. It is likely that the reduced levels of whole-blood PrP^c in neurological controls is a consequence of the reduced PrP^c levels found in RBCs and PLTs in this group. To some extent the low levels of PLT PrP^c may reflect neurological disease because PrP^c levels were low in PLT samples from CJD and lowest in neurological controls but not in healthy adult controls. We are unable though to speculate on the reasons for this.

We also considered the possibility that increases in sCJD plasma PrP^c levels may be a consequence of its release from PLTs, because PLTs are known to express PrP^c on the cell surface and to store PrP^c in alpha granules, which are released into plasma in a soluble form on PLT activation.[24,32] To support this theory, we have found a significant reduction in the PLT PrP^c concentration in sCJD patient samples compared to healthy adults, implying that PLTs may have shed PrP^c into plasma. The expression of PrP on PLTs of vCJD and sCJD as determined by flow cytometry was elevated compared with healthy adult and neurological controls, and although this elevation did not reach significance, perhaps this represents up regulation of PrP before its release into plasma. Investigations into cell-surface PrP on PLTs did not implicate blood sample storage and transit as a possible cause for increased cell-surface PrP; they found cell-surface PrP on PLTs decreased with sample age.

Flow cytometry analysis demonstrated significantly less PrP expression on RBCs of neurological controls than healthy controls. This finding supports that of a reduced concentration of RBC PrP^c in this group found by DELFIA. These results would appear to indicate that there is less PrP^c per RBC compared with other groups. We are unable to explain the reason behind the finding of this reduced expression of PrP on RBCs. We did not find any evidence of a reduction in the expression of PrP on lymphocytes of patients with sCJD when compared to neurological controls in contrast to a previous report.[33] We used both 4F2 and 3F4 anti-PrP MoAbs together for these flow cytometry studies, however, whereas the previously reported study used 3F4 alone, which does not to bind to human RBCs.[29] It could be possible that MoAb 3F4 might show reduced binding to PrP^c on lymphocytes in some clinical conditions if PrP^c expression were altered or PrP^c interacted with other membrane components because of lymphocyte activation.[34,35] PrP on RBCs, PLTs, and lymphocytes was removed to background levels after pK treatment in all clinical and control groups, indicating the absence of any detectable cellular expression of abnormal disease associated forms of PrP. Our findings indicate that a reduction

in concentration of whole-blood PrP^c may be common in vCJD and other neurological diseases but not sCJD. An elevated level of plasma PrP^c may be common in sCJD. These differences between groups appear genuine, presumably reflecting differences in the disease process in the patients in these particular groups and not an artifact of age, specimen collection, storage, or analysis. Despite the significance of differences between groups, the variations in values are large and there is considerable overlap between CJD groups and control groups, which rules out the exploitation of these differences in whole blood and plasma in screening strategies. These studies expose the limitations in the use of blood PrP^c levels as a diagnostic tool. They illustrate, however, important observations on the distribution of PrP^c in the peripheral blood of CJD patients and the potential of DELFIA-based PrP assays in clinical practice. The analytical sensitivity of DELFIA-based assays used here represents a significant step toward the development of DELFIA for the detection of PrP^{Sc}, which is a much more reliable indicator of infection and is directly associated with infectivity. The current study emphasizes the need to develop assays for its detection in blood.[36,37]

ACKNOWLEDGMENTS

We acknowledge the support of patients and families affected by vCJD and sCJD for providing consent for these studies. We also thank apheresis blood donors and staff at the Scottish National Blood Transfusion for providing control samples.

REFERENCES

1. Prusiner SB. Novel proteinaceous infectious particles cause scrapie. Science 1982;216:136-44.
2. Will RG, Ironside JW, Zeidler M, et al. A new variant of Creutzfeldt-Jakob disease in the UK. Lancet 1996;347:921-5.
3. Hill AF, Zeidler M, Ironside J, Collinge J. Diagnosis of new variant Creutzfeldt-Jakob disease by tonsil biopsy. Lancet 1997;349:99-100.
4. Wadsworth JD, Joiner S, Hill AF, et al. Tissue distribution of protease resistant prion protein in variant Creutzfeldt-Jakob disease using a highly sensitive immunoblotting assay. Lancet 2001;358:171-80.
5. Houston F, Foster JD, Chong A, et al. Transmission of BSE by blood transfusion in sheep. Lancet 2000;356:999-1000.
6. Hunter N, Foster J, Chong A, et al. Transmission of prion diseases by blood transfusion. J Gen Virol 2002;83:2897-905.
7. Llewelyn CA, Hewitt PE, Knight RS, et al. Possible transmission of variant Creutzfeldt-Jakob disease by blood transfusion. Lancet 2004;363:417-21.
8. Peden AH, Ritchie DL, Bell JE, Ironside JW. Preclinical vCJD after blood transfusion in a PRNP codon 129 heterozygous patient. Lancet 2004;364:527-9.
9. Brown P, Cervenakova L, McShane LM, et al. Further studies of blood infectivity in an experimental model of transmissible spongiform encephalopathy, with an explanation of why blood components do not transmit Creutzfeldt-Jakob disease in humans. Transfusion 1999;39:1169-78.
10. Brown P, Rohwer RG, Dunstan BC, et al. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. Transfusion 1998;38:810-6.
11. Klein MA, Frigg R, Flechsig E, et al. A crucial role for B cells in neuroinvasive scrapie. Nature 1997;390:687-90.
12. Klein MA, Frigg R, Raeber AJ, et al. PrP expression in B lymphocytes is not required for prion neuroinvasion. Nat Med 1998;4:1429-33.
13. Mabbott NA, Bruce ME. The immunobiology of TSE diseases. J Gen Virol 2001;82:2307-18.
14. Mabbott NA, Farquhar CF, Brown KL, Bruce ME. Involvement of the immune system in TSE pathogenesis. Immunol Today 1998;19:201-3.
15. Prinz M, Heikenwalder M, Junt T, et al. Positioning of follicular dendritic cells within the spleen controls prion neuroinvasion. Nature 2003;425:957-62.
16. Haik S, Faucheux BA, Sazdovitch V, et al. The sympathetic nervous system is involved in variant Creutzfeldt-Jakob disease. Nat Med 2003;9:1121-3.
17. Hunter N. Pathogenesis of TSEs in sheep: experimental studies and natural disease. In: Prion diseases: from basic research to intervention concepts. International Prion Conference; 2003; Gasteig, Munich.
18. Head MW, Ritchie D, Smith N, et al. Peripheral tissue involvement in sporadic, iatrogenic, and variant Creutzfeldt-Jakob disease: an immunohistochemical, quantitative, and biochemical study. Am J Pathol 2004; 164:143-53.
19. Bruce ME, McConnell I, Will RG, Ironside JW. Detection of variant Creutzfeldt-Jakob disease infectivity in extraneural tissues. Lancet 2001;358:208-9.
20. Brown P, Cervenakova L, Diringer H. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease. J Lab Clin Med 2001;137:5-13.
21. Brown P. Creutzfeldt-Jakob disease: blood infectivity and screening tests. Semin Hematol 2001;38(4 Suppl 9):2-6.
22. Cervenakova L. The safety of human blood: experimental TSE/prion infectivity studies. Transfus Clin Biol 2001;8: 260.
23. MacGregor I, Hope J, Barnard G, et al. Application of a time-resolved fluoroimmunoassay for the analysis of normal prion protein in human blood and its components. Vox Sang 1999;77:88-96.
24. Bessos H, Drummond O, Prowse C, et al. The release of prion protein from platelets during storage of apheresis platelets. Transfusion 2001;41:61-6.
25. Volkel D, Zimmermann K, Zerr I, et al. Immunochemical determination of cellular prion protein in plasma from

healthy subjects and patients with sporadic CJD or other neurologic diseases. Transfusion 2001;41:441-8.

26. Ironside JW, Head MW, Bell JE, et al. Laboratory diagnosis of variant Creutzfeldt-Jakob disease. Histopathology 2000;37:1-9.
27. Budka H, Aguzzi A, Brown P, et al. Neuropathological diagnostic criteria for Creutzfeldt-Jakob disease (CJD) and other human spongiform encephalopathies (prion diseases). Brain Pathol 1995;5:459-66.
28. Farquhar CF, Dickinson AG. Prolongation of scrapie incubation period by an injection of dextran sulphate 500 within the month before or after infection. J Gen Virol 1986;67:463-73.
29. Barclay GR, Houston EF, Halliday SI, et al. Comparative analysis of normal prion protein expression on human, rodent, and ruminant blood cells by using a panel of prion antibodies. Transfusion 2002;42:517-26.
30. Barclay GR, Hope J, Birkett CR, Turner ML. Distribution of cell-associated prion protein in normal adult blood determined by flow cytometry. Br J Haematol 1999;107:804-14.
31. Tukey JW. Exploratory data analysis. Reading (MA): Addison-Wesley; 1977.
32. Perini F, Vidal R, Ghetti B, et al. PrP27-30 is a normal soluble prion protein fragment released by human platelets. Biochem Biophys Res Commun 1996;223:572-7.
33. Ratzka P, Dohlinger S, Cepek L, et al. Different binding pattern of antibodies to prion protein on lymphocytes from patients with sporadic Creutzfeldt-Jakob disease. Neurosci Lett 2003;343:29-32.
34. Li R, Liu D, Zanusso G, et al. The expression and potential function of cellular prion protein in human lymphocytes. Cell Immunol 2001;207:47.
35. Mattei V, Garofalo T, Misasi R, et al. Prion protein is a component of the multimolecular signaling complex involved in T cell activation. FEBS Lett 2004;560:14.
36. Safar J, Wille H, Itri V, et al. Eight prion strains have PrP(Sc) molecules with different conformations. Nat Med 1998;4:1157-65.
37. Bellon A, Seyfert-Brandt W, Lang W, et al. Improved conformation-dependent immunoassay: suitability for human prion detection with enhanced sensitivity. J Gen Virol 2003;84:1921-5. ◘

別紙様式第2

研究報告調査報告書

識別番号		年　月　日	登録番号		年　月　日

報告区分	研究報告			(厚生労働省処理欄)
一般的名称	胎盤加水分解物	研究報告又は外国における措置の公表状況		
販売名(企業名)	ラエンネック (日本生物製剤)			
研究報告又は外国での措置の概要	問題点(医師および薬剤師向け情報：凝固因子およびプリオンに関する情報) 2005年1月10日，ZLB Behringはポールエーリッヒ研究所に，後に変異型クロイツフェルト・ヤコブ病を発症したフランス人女性ドナーの血漿は1996年に中間製品において使用されていたことを通知した。中間製品はHaemate HS/P 1000(バッチ番号5676641)の製造に用いられた。Haemateは血友病Aおよび後天性第VIII因子欠損に関連した出血の予防および治療などに用いられる。当該バッチは1997年にCenteonによってドイツ市場に出回った。しかしポール・エールリッヒ研究所はフランスの血液ドナーセンターでの遡及的に認識され始めたドナー選択における不適合が確認された後バッチを回収した。続いてCenteonはこれらバッチの自主回収を行った。ドイツにおいて販売された1494個のうち1269個は既に使用されているようであり，225個が返品された。このバッチの使用による患者のvCJD感染リスクは極めて低いと考える。加えて，血漿由来製品によるvCJDの感染確定例または疑い例は発生していない。ポールエーリッヒ研究所が実施したこのバッチについてのリスク計算などについて記載されている。			使用上の注意記載状況等 記載なし。 その他の参考事項
報告企業の意見	特に、当社の製品であるラエンネックとの関連が認められない為、問題なしと思われます。	処置と今後の対応	現状維持とします。	

12

back

Information for Physiscians and Pharmacists

Paul-Ehrlich-Institut
Federal Agency for Sera and Vaccines

Information on Coagulation Factors and Prions

(pdf-version for download; new Browser)

On 10th January 2005, ZLB Behring informed the Paul-Ehrlich-Institut in Langen, Germany that the plasma of a French woman donor, who later developed vCJD, was used in an intermediate product in 1996. The intermediate product was used to manufacture Haemate HS/P 1000 (Batch No 5676641). Haemate is used in the prophylaxis and therapy of bleeding relating to Haemophilia A and acquired factor VIII deficiency, in the therapy of patients with antibodies against factor VIII, and the prophylaxis and therapy of bleeding relating to Willebrand syndrome.

The batch referenced was introduced into the German market by the then license holder, Centeon, in 1997. However, the PEI had withdrawn the batch release in November 1997 after detecting non-conformities in the donor selection in the French blood donor centre that had become known in retrospect. Following this, Centeon recalled the batches at its own responsibility. 1269 of 1494 packages marketed in Germany had apparently already been consumed, 225 were sent back.

The risk of infection of patients with vCJD by using the batch in question is to be considered as extremely low. In addition, no confirmed or suspected case of transmission of vCJD by plasma derivatives has occurred. The Paul-Ehrlich-Institut has carried out a risk calculation for the batch concerned on the basis of the following parameters:

- the amount of the donor's plasma used
- the size of the plasma pool in which the plasma donation was used
- the amount of plasma processed for a single dose (package)
- an assumed pathogen concentration based on data from animal experiments
- the experimentally examined capacity of the purification steps to remove prions during the manufacturing procedure of factor VIII products

According to this calculation, the possibly remaining residual amount of infectivity in a single dose of the Haemate HS/P 1000 batch in question is considered as very low (4.1×10^{-4} IU_{50}/ package to 4.1 x $^{-5}$ IU_{50}/ package).

In other words, one out of 2,500 to 25,000 packages could contain one theoretical infective unit of prions. Since these considerations indicate that only every 2,500th to 25,000th package may be contaminated, there is a high probability that none – or at least only a small percentage – of the 1269 packages contain residual amounts of infected material. Therefore, the risk is still small even for patients who received several packages from this batch.

At a European level, extensive discussions have been underway concerning the safety of plasma-derived products for several years. A position paper of the Committee for Medicinal Products for Human Use (CHMP (formerly CPMP)) at the European Agency for the Evaluation of Medicinal Products (EMEA) dated June 2004 states that in using French plasma, too, the safety margin regarding prion infection is sufficient. These evaluations are being continuously adapted to the state of the art (http://www.emea.eu.int/pdfs/human/press/pos/287902rev1.pdf).

If you need additional information, please contact Dr. Susanne Stöcker
Phone: +49 6103 / 77 1030 or Fax: +49 6103 / 77 1262 or E-Mail: 'pressestelle'

別紙様式第 2-1
番号 20

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 2005年4月18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	①ポリエチレングリコール処理抗破傷風人免疫グロブリン ②乾燥抗破傷風人免疫グロブリン		研究報告の公表状況	Transfusion 45(4)500-503, 2005	公表国 アメリカ	
販売名（企業名）	①テタノブリン－IH（ベネシス） ②テタノブリン（ベネシス）					

研究報告の概要	使用上の注意記載状況・その他参考事項等
1986年から1990年の間に米国メリーランド州ボルチモアのFACTS（Frequency of Agents Communicable by Transfusion study）に登録された患者406人の血清について、手術前及び手術6箇月後のHHV-8抗体がlytic-antigen免疫蛍光法で測定された。手術前にHHV-8抗体陽性率は11.3%であった。輸血時にHHV-8抗体陰性であった患者284人のうち2人が陽転化した。これらの患者は血液製剤を受けており、輸血を受けなかったHHV-8抗体陰性の患者に陽転化は見られなかった。もし陽転化の輸血が原因であるとすると、輸血による製剤当りのHHV-8伝播リスクは0.082%となる。米国における血液製剤を介したHHV-8伝播を示唆する最初の報告である。	代表としてテタノブリン－IHの記載を示す。 2. 重要な基本的注意 (1) 本剤の原材料となる血液については、HBs抗原、抗HCV抗体、抗HIV-1抗体、抗HIV-2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高力価の破傷風抗毒素を含有する血漿を原料として、Cohnの低温エタノール分画で得た画分からポリエチレングリコール4000処理、DEAEセファデックス処理等により抗破傷風人免疫グロブリンを濃縮・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。

報告企業の意見	今後の対応
輸血により、HHV-8が伝播したとの報告である。 血漿分画製剤からのHHV-8伝播の事例は報告はされていない。万一原料血漿にHHV-8が混入したとしても、BHV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。

テタノブリン

13