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Age-related expression of the cellular prion protein in human peripheral blood leukocytes

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ABSTRACT

Background and Objectives. **Creutzfeldt-Jakob disease typically affects older patients, yet victims of new-variant Creutzfeldt-Jakob disease (nvCJD) are unusually young. Because the cellular prion protein PrP^C is required for disease development, we investigated age-dependent variability in cell surface PrP^C expression on various subclasses of human peripheral blood leukocytes (PBL) as a possible susceptibility factor.**

Design and Methods. **Three age groups of healthy individuals (mean ages: 6, 33 and 68) were studied by two-color FACS analysis of PBL with fluorescent monoclonal antibodies to PrP^C and to the lineage markers CD3, CD19, CD4, and CD8. For each subclass marker, surface PrP^C levels were expressed as mean fluorescence intensity ratios (MFIR) by dividing the geometric mean of the fluorescence of each test antibody by the geometric mean of its isotype-matched control antibody. PrP^C expression levels in each age and lineage group were compared using appropriate non-parametric tests.**

Results. **We found significant age-related differences in PrP^C expression on lymphocytes (p=0.0004). The elderly expressed significantly higher levels than children (p=0.0006) and adults (p=0.0009). PrP^C expression was also significantly higher in $CD3^+$ compared to $CD19^+$ (p=0.0004) and in $CD8^+$ compared to $CD4^+$ lymphocytes (p=0.0044).**

Interpretation and Conclusions. **If PrP^C expression on PBL were a significant susceptibility factor for nvCJD, young persons would display higher levels. Instead, the elderly expressed the highest amounts of PrP^C on PBL. This argues against the hypothesis that variability in cell surface expression of PrP^C in PBL contributes to the exquisite susceptibility of the young to nvCJD.**
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Key words: prion protein, age-dependent expression, new variant CJD, peripheral blood leukocytes

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Prion diseases or transmissible spongiform encephalopathies (TSE) are neurologic disorders caused by transmissible pathogens termed prions. While the prototype of all prion diseases, scrapie of sheep and goats, has been known for more than two centuries, a new form of animal prion disease designated bovine spongiform encephalopathy (BSE) was recognized in 1986 and has since developed into an epizootic.[1,2] Creutzfeldt-Jakob disease (CJD) is one of the human TSE. It occurs sporadically (incidence: $1\text{-}2\times10^{-6}$) but in 10% to 15% of all cases it is transmitted genetically as an autosomal dominant trait. Gerstmann-Sträussler-Scheinker syndrome (GSS) and fatal familial insomnia (FFI) are also human TSE, which are transmitted as dominant traits.

One of the hallmarks of prion diseases is the accumulation in the central nervous system (CNS) of an abnormal isoform (PrP^{Sc}) of the host encoded prion protein (PrP^C).[3-5] Although the exact physical nature of the transmissible agent remains controversial, a growing body of experimental data supports the 'protein only' hypothesis, which postulates that the agent is devoid of nucleic acid and consists solely of an abnormal conformer of the cellular prion protein, PrP^C.[6,7]

By virtue of its location on the outer surface of cells, anchored by phosphatidylinositol glycolipid,[8] PrP^C is a candidate for a signaling, cell adhesion or (less likely) transport function. Various functions, including copper binding, participation in lymphocyte activation, and Purkinje cell long-term survival, have been ascribed to PrP^C.[9-12] PrP^C is expressed on many cell types, including neurons,[13] astrocytes[14] and lymphocytes[10] and appears to be developmentally regulated during mouse embryogenesis.[15] Although PrP^C is predominantly found in brain tissue, high levels are also present in heart, skeletal muscle and kidney whereas it is barely detectable in the liver.[16]

PrP^C is required for susceptibility to prions, and mice homozygous for *Prnp* null alleles cannot be infected with prions.[17,18] The lymphoreticular system plays an important role in prion replication.[19-21] Prion replication in lymphoid organs always precedes replication in the CNS (even after intracerebral administration of prions), and immunodeficient mice lacking mature B-lymphocytes do not develop disease following intraperitoneal inoculation.[22-24]

Splenic B and T-lymphocytes of scrapie-infected mice acquire prion infectivity early in the course of the disease.[25] Because both PrP^c and the disease isoform PrP^{Sc} are found in peripheral lymphoid tissues derived from pre-symptomatic CJD patients, the safety of blood products has been questioned.[26,27]

The emergence of a new variant form of Creutzfeldt-Jakob disease (nvCJD) in young people in the UK raised the possibility that BSE may spread to humans by dietary exposure.[28,29] This frightening scenario has been supported recently by experimental evidence indicating that the agent causing BSE is indistinguishable from the nvCJD agent.[30-33] Susceptibility factors for nvCJD such as the precise role of leukocytes in disease progression and the minimum dose of BSE necessary to infect humans orally are not well understood. Young age in association with homozygosity for methionine at *PRNP* (the gene encoding the prion protein) codon 129 (all victims to date were homozygous for methionine at this locus) and UK residence (all but one French victim to date were UK residents), are the only factors that appear to predispose individuals to nvCJD. Prediction of the scale of the disease is difficult, in the absence of good understanding of the epidemiology and susceptibility factors for nvCJD, with anywhere between 75 and 80,000 cases postulated.[34-36]

In view of the young age of nvCJD victims, we have investigated age-dependent differences in peripheral expression of PrP^c, under the assumption that enhanced expression may render younger individuals more susceptible to acquired encephalopathy. We have also analyzed PrP^c surface immunoreactivity in various blood cell lineages. It is our hope that detailed study of the patterns and of the factors controlling PrP^c expression in peripheral tissues will give further clues to the physiological function of PrP^c and the role of peripheral blood leukocytes in the transport and replication of the TSE agent from the periphery to the CNS.

Design and Methods

Subjects

To study age-related PrP^c expression, peripheral blood was obtained from 31 children at the University Children's Hospital of Zürich. These children were between 0.2 and 13 years old (mean age: 6.3). Children affected by infectious or other diseases that would affect the composition of PBL were excluded from this study. Parental consent was obtained. The study was presented to, and approved by, the Hospital Ethical Committee. Blood was also obtained from 31 healthy adult donors from the blood donation center (Blutspendezentrum) of the city of Zürich. These donors were aged 26-42 (mean age of 33 years). All donors had tested negative for HIV and hepatitis and had normal hematology profiles. Finally, we analyzed the blood of 32 elderly patients aged 62-83 years (mean age of 68 years). Twenty of these were blood donors, while the remaining were patients of the Surgery Department of the University Hospital of Zürich treated for minor injuries. PrP^c expression in CD19$^+$ and in CD3$^+$ lymphocytes was studied using 20 adult healthy blood donors aged 21-55 (mean age of 33). PrP^c expression in CD4$^+$ versus CD8$^+$ T-lymphocytes was studied in blood from 34 further donors aged 22-50 (mean age of 35) (Table 1).

Sample preparation and immunofluorescence staining

Peripheral blood was drawn by venipuncture into 5 mL EDTA tubes (Vacutainer, Becton and Dickinson, Pasadena, CA, USA) and stored at 4°C prior to processing, for no longer than four hours. A complete blood count including automated differential was performed on a Technicon H3RTX hematometer (Bayer, Germany) prior to isolation of PBL by density gradient centrifugation. Peripheral blood mononuclear cells (PBMC) were obtained by Lympholyte-H gradient (density =1.07 g/L CedarLane Labs. Ltd., Ontario, Canada) centrifuged at 800 g for 15 minutes at room temperature. Cells were washed with FACS buffer (PBS, 2% heat inactivated fetal calf serum (HIFCS), 0.05% sodium azide), and incubated for 15 minutes on ice with the patient's own plasma to block Fc receptors. PBMC were washed again in FACS buffer, and re-counted with the hematometer. Cells were then resuspended in FACS buffer and adjusted to a concentration of 2.5×10^6 cells/mL. Saturating amounts of monoclonal antibodies were added to 250×10^3 PBMC in each tube, and incubated for 20 minutes on ice. To stain for PrP^c mouse monoclonal antibodies 3F4 (IgG2a) (37) and 6H4 (IgG1)[38] were used, at 1-5 µg and 2.8 µg per tube, respectively. Fluorescent detection was accomplished with FITC-conjugated anti-mouse F(ab)$_2$ fragments (Serotec, Oxford, UK) at 0.25 µg/tube. Simultane-

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Table 1: Demographic and hematological characteristics of test persons

Groups	*Median Age (range)*	*Males/ Females*	*WBC$\times10^3$/µL Median (range)*	*Hemoglobin g/100 mL Median (range)*	*Platelets $\times10^3$/µL Median (range)*	*Lymphocytes %*	*Monocytes %*
Adult, n=31	33 (26-42)	22/9	5.2 (2.6-11.0)	13.3 (5.4-17.0)	219 (76-395)	34.4 (14.8-47.4)	6 (4.3-22.0)
Children, n=31	6.0 (0.2-13.0)	18/13	8.4 (5.0-15.7)	12.1 (4.1-15.1)	297 (60-588)	42.1 (11.6-85.0)	6.4 (3.1-10.6)
Elderly, n=32	66.5 (61-83)	19/13	5.5 (3.2-12.5)	12.1 (6.0-15.8)	234 (115-644)	28.3 (10.9-40.6)	6.5 (4.9-10.6)
CD3+,CD19+, n=20	31 (21-55)	10/10	5.4 (3.4-11.5)	12.5 (7.3-16.0)	220 (123-395)	31.6 (14.6-41.0)	6.4 (4.1-8.9)
CD4+,CD8+, n=34	35 (22-50)	24/10	5.0 (2.6-11.0)	13.1 (4.4-15.8)	226 (76-471)	35.0 (19.7-48.8)	6.1 (3.9-22.0)

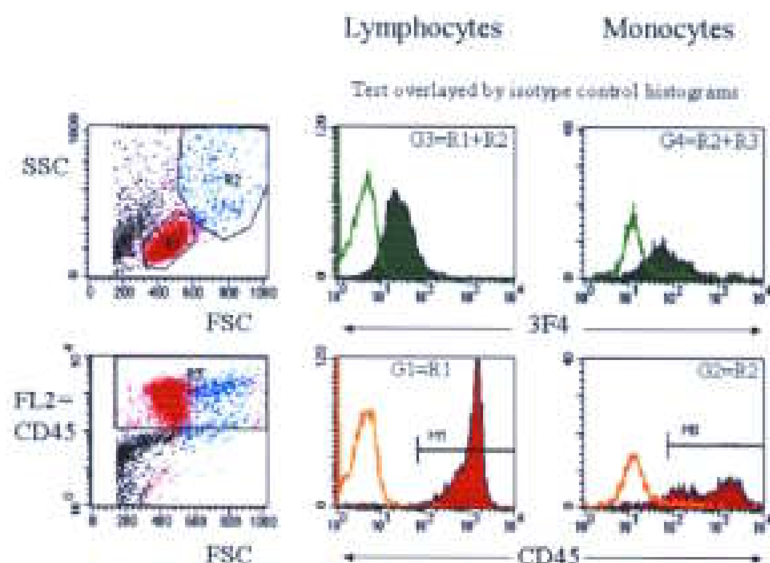

Figure 1. Histogram and dot plot analysis of human PBMC two colour FACS for PrPc F4) and lineage markers. The histograms shown were derived from blood of a 22 years old adult blood donor. GATING: Lymphocyte (R1) and monocyte (R2) gates based on forward (FSC) and side scatter (SSC) of PBMC. The gate denominated R3 represents all CD45 (leukocyte common antigen) positive leukocytes. LYMPHOCYTES: 3F4 and IgG2a control antibody histograms (FL1) represent events that satisfy the G3=R1+R3 gate i.e. CD45 positive lymphocytes. MFIR= 3F4MFI/IgG2aMFI = 27/3.6=7.5. Histograms for lymphocyte CD45 and IgG1 for the same sample (FL2), gated on G1=R1 alone. MONOCYTES: 3F4 and IgG2a control antibody histograms (FL1) represent events that satisfy the G4=R2+R3 gate i.e CD45 positive monocytes. MFIR= 56/12.5= 4.48. Histograms for lymphocyte CD45 and IgG1 for the same sample (FL2), gated on G2=R2 alone.

ous staining with various leukocyte surface markers was performed using commercial antibodies: phycoerythrin labeled anti-CD45 (Becton and Dickinson), phycoerythrin labeled anti-CD3 and anti-CD19 (Caltag Laboratories, USA) and Cy5 labeled anti-CD4 and anti-CD8 (Coulter-Immunotech, France). For determination of background fluorescence levels, isotype control antibodies were used at the same concentration as the test antibodies. Samples from 3 to 6 patients from each group were analyzed in each experimental session to minimize variation between experiments.

Flow cytometry

Flow cytometry was performed on a FACS Calibur (Becton and Dickinson) equipped with a 488 nm emitting argon laser. The instrument was calibrated for three-color cytometry before each experiment using Calibrite beads (Becton and Dickinson) to minimize spectral overlap between fluorochromes and facilitate compensation. Forward (FSC) and side scatters (SSC) were collected as linear signals and all fluorescent emissions on a four-decade logarithmic scale. Ten thousands events in the leukocyte gate were acquired in list mode using CellQuest software (Becton and Dickinson). Every effort was made to acquire all data using the same voltage settings for the fluorescence detectors. The staining intensity was expressed as mean fluorescence intensity ratio (MFIR): MFIR values were calculated by dividing the geometric mean of the fluorescence of each test antibody by the geometric mean of its isotype-matched control antibody. Because the fluorescence of each relevant antibody and of its respective control were recorded using identical instrument settings, MFIR values are independent of variations in signal amplification. PrP^c histograms were calculated using cell populations gated on a combination of the lymphocyte or monocyte gate (R1 and R2 respectively) based on forward scatter, and combined with the CD45 positive gate (R3). For CD45 or other lineage markers, histograms were gated on the lymphocyte or monocyte gates alone (R1 or R2) (Figure 1).

Statistical analysis

All data were analyzed using StatView 5.0.1 (SAS Institute Inc, USA). Data were presented as median values and interquartile ranges. Due to the asymmetric distribution of the data, non-parametric tests were used to compare MFIR in each group. The Mann-Whitney U test and the Kruskal-Wallis tests were employed for unpaired comparisons between two or more age groups respectively. The Wilcoxon signed rank test was employed for paired comparisons such as those for PrP^c MFIR between $CD3^+$ and $CD19^+$, or $CD4^+$ and $CD8^+$ lymphocytes derived from the same patients. The criterion for statistical significance was defined as $p<0.01$ for the Kruskal-Wallis and Wilcoxon signed rank tests and $p<0.0033$ (0.01/3 groups) for the Mann-Whitney test.

Results

The demographic and hematologic characteristics of the donors are listed in Table 1. Children had significantly higher white blood cell counts then both elderly and adults, in accordance with published observations ($p<0.001$, Kruskal-Wallis).[39,40] The reasons behind this phenomenon are unclear but may reflect a higher activity of the immune system in the young. No other differences of significance were observed between the blood donor groups.

Leukocyte expression of PrP^c and of CD45 in various age groups

Age-related differences in surface PrP^c immunoreactivity of lymphocytes and monocytes were examined. For lymphocytes, median MFIR was found to be 5.9 for children, 5.1 for adults, and 8.7 for the elderly. Statistical analysis using the Kruskal-Wallis test evidenced highly significant age-related differences in

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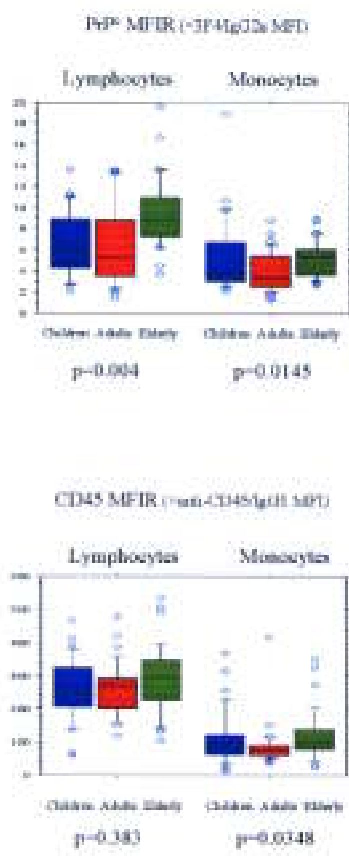

Figure 2. a: Surface PrPc immunoreactivity of human PBMC from different age groups. Box plot analysis of age related PrPc lymphocyte and monocyte MFIR for children, adult and elderly groups. Lines in the middle of each box represent the median MFIR for each age group. The edges of each box represent the 25th and 75th percentile. 50% of all observations are within the box. Values outside the interquartile range represented by open circles. Kruskal-Wallis derived p values for comparisons of the MFIR between all age groups indicated. Significance threshold at p<0.01. There is a clear-cut increase in PrPC surface expression in lymphocytes of elderly persons. b: Surface CD45 immunoreactivity of human PBMC from different age groups. Box plot analysis of age related PrPc lymphocyte and monocyte MFIR for children, adult and elderly groups.

the average lymphocyte surface PrP^C MFIR (p=0.0004) (Figure 2a and Table 2). To explore the differences observed, further comparisons between pairs of age groups were made employing the Mann-Whitney U test. The elderly had significantly more surface MFIR for PrP^C than children (p=0.0006) or adults (p=0.0009), while differences between children and adults were not significant (p=0.64) (Table 2). We then asked whether the differences observed might represent a systematic artifact due to the different origin of the samples (blood donation center versus Children's Hospital), which may have affected the metabolic state of the PBL. We, therefore, we performed the same type of normalized analysis using the CD45 leukocyte common antigen marker. However, no such age-related differences were observed in the expression levels of CD45 (p=0.384, Figure 2b).

Table 2; Analysis of age-related MFIR of PrP^c in lymphocytes and monocytes.

	PrP^c MFIR in lymphocytes Mean±SE	PrP^c MFIR in lymphocytes Median (range)	PrP^c MFIR in monocytes Mean±SE	PrP^c MFIR in monocytes Median (range)
Children	6.43±0.5	5.9 (2.0-13.6)	5.2±0.6	3.6 (2.0-18.0)
Adults	6.34±0.6	5.1 (1.4-13.6)	3.9±0.3	3.3 (1.3-8.8)
Elderly	9.5±0.6	8.7 (3.7-19.6)	5.1±0.3	5.2 (2.7-8.8)
All groups	p = 0.0004*		p = 0.0145	
Elderly-children	p = 0.0006#		p = 0.1236	
Elderly-adults	p = 0.0009#		p = 0.0039	
Adults-children	p = 0.64		p = 0.1699	

**Kruskal-Wallis test used to compare mean MFIR between all age groups, significant when p< 0.01; #Mann-Whitney test used for comparisons between pairs of groups, significant when p<0.0033.*

Table 3: Gender comparison for lymphocyte and monocyte PrPc and CD45 MFIR.

	Lymphocytes PrP^c MFIR	Lymphocytes CD45 MFIR	Monocytes PrP^c MFIR	Monocytes CD45 MFIR
Children (13 F, 18 M)	p=0.32	p= 0.52	p=0.84	p=0.09
Adults (9 F, 22 M)	p=0.29	p= 0.27	p=0.63	p=0.38
Elderly (13 F, 19 M)	p=0.34	p= 0.53	p=0.39	p=0.45

Mann Whitney test used to compare PrPc and CD45 MFIR for gender within each age group. Significant gender differences when p< 0.01. F = females; M = males.

Because monocytes are also known to express PrP^C, we investigated PrP^C MFIR of monocytes. Median MFIR in the various age groups were 3.6 for children, 3.9 for adults, and 5.2 in the elderly. Therefore, the same trend of increased PrP^C surface expression levels was observed in monocytes of the elderly. Statistical analysis by the Kruskal-Wallis test showed that this trend fell just short of the significance threshold adopted in the present study (p=0.0145) (Figure 2a and Table 2). MFIR for monocyte surface CD45 was employed as an internal control of the monocyte analysis, and showed a pattern similar to that of lymphocyte surface CD45 with no significant age-related variation (p=0.0348, Figure 2b). Further confidence in the validity of the results reported above derives from the observation that the standard errors for PrP^C MFIR in each age group are small, for both lymphocytes and monocytes. In contrast the variation in MFIR between individuals is much larger, reflecting differential expression of PrP^c on the surface of lymphocytes and monocytes. No gender differences were observed in the surface immunoreactivity of either PrP^c or CD45 within each age group for either lymphocytes or monocytes (Table 3).

When individuals of all age groups were analyzed as a pool, the mean MFIR for surface PrP^C in monocytes was significantly lower than that found in lymphocytes (n=94, Wilcoxon signed rank test:

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Table 4. MFIR and MFI as measures for PrPc cell surface immunoreactivity.

All age groups (n=94)	*Lymphocytes Mean±SE*	*Monocytes Mean±SE*	*p Wilcoxon paired*
MFIR = 3F4 MFI/IgG2a MFI	7.4±0.37	4.7±0.263	<0.0001*
3F4 MFI	27.9±1.4	67.7±3.39	<0.0001*

**Wilcoxon signed rank test for comparisons between paired groups was used to statistically compare the MFIR or MFI between lymphocytes and monocytes. MFIR comparison reveals that lymphocytes have significantly higher PrPc surface immunoreactivity compared to monocytes. MFIR unlike MFI is a measure of immunoreactivity independent of cell size. Significant when p<0.01. SE: standard error.*

Table 5. Mean fluorescence intensity ratio of PrPc in different lymphocyte subsets.

Cell type	*Mean±SE*	*Median (range)*	*p Wilcoxon test*
CD3+, n=20	7.2±0.6	7.0 (2.9-11.9)	
CD19+, n=20	4.7±0.45	4.1 (1.8-8.6)	0.0004*
CD8+ high, n=34	8.0±0.74	7.8 (1.7-19.0)	
CD4+, n=34	6.6±0.6	7.2(1.9-14.5)	0.0044*

**Significant differences in MFIR for CD3± versus CD19± and CD8± versus CD4+ when p< 0.0*

p<0.0001). Here we encountered an apparent discrepancy: when the mean fluorescence intensity (MFI) for PrP^C was investigated, monocytes gave consistently higher MFI values than lymphocytes for both PrP^C and isotype matched control antibodies (Table 4). However, comparisons between monocytes and lymphocytes are confounded by the fact that the average membrane surface of monocytes is larger than that of lymphocytes. Moreover, monocytes differ from lymphocytes in their expression of surface molecules that may bind non-specifically to antibodies, such as Fc and complement receptors. The MFIR measurement employed in this study minimizes the impact of background binding, is independent of cell size, and reflects the true density of a given antigen per unit of cell surface more accurately than simple measurement of fluorescent intensity. Therefore, MFIR is, in our view, an appropriate index of PrP^C expression.

PrP^c expression in leukocyte subpopulations

Various leukocyte subpopulations were also analyzed for surface PrP^c immunoreactivity. Results for B (CD19$^+$) and T (CD3$^+$) lymphocytes and CD8^{high} compared to CD4$^+$ T-lymphocytes are summarized in Table 5. Briefly, CD3$^+$ lymphocytes had significantly higher PrP^c mean MFIR than CD19$^+$ lymphocytes and cytotoxic T-lymphocytes (CD8^{high}) significantly more than CD4$^+$ T lymphocytes.

All results presented above were based on the use of monoclonal antibody 3F4 raised against purified human prion amyloid fibrils, which recognizes residues 109-112 of PrP^C in hamsters and humans. To validate the study further, we repeated the analyses presented above in a small subgroup of test subjects with monoclonal antibody 6H4. This antibody was raised against recombinant bovine PrP^C produced in *E. coli*, and recognizes residues 144-152 of human PrP^c. FACS analysis of PBL stained with antibody 6H4 gave results very similar to those obtained with antibody 3F4 (data not shown).

Discussion

A large proportion of the UK population and many individuals in Europe have in all likelihood had dietary exposure to the BSE agent. In the thirteen years since BSE first appeared and six years since the peak of the BSE epidemic in the UK, 44 confirmed and 47 possible cases of nvCJD have been reported, all affecting young people (mean age 28 years). All victims to date were homozygous for methionine at the polymorphic *PRNP* codon 129. These facts suggest that individual susceptibility factors may modulate the ability of BSE to infect humans. PrP^C is necessary for prion disease development[17,41,42] and lymphocytes can be infected with prions in a PrP^c dependent manner.[25] Overexpression of wild-type PrP^C in transgenic mice significantly shortens the incubation time of the disease upon inoculation with scrapie prions.[43] Based on the above observations we hypothesized that age-dependent variability in peripheral blood leukocyte surface PrP^C might explain the age spectrum of patients affected by nvCJD. The data presented in this study, however, allow us to reject this original hypothesis. Children and young adults did not have higher surface PrP^C than elderly people. In contrast, a very significant increase in surface PrP^C immunoreactivity was found in lymphocytes of elderly subjects. We cannot exclude the possibility that a different turnover of PrP^C in old age could explain the differences observed. We have no specific reason to postulate age-related differences in PrP^c turnover, but examples of erroneous RNA processing in age with functional implications have been described. Moreover, even if this were the case with PrP^c, our conclusions with respect to nvCJD would not be affected.

Sporadic CJD, which accounts for the vast majority of CJD cases (> 80%), is a disease of old age, typically appearing in the 7th decade of life. It occurs spontaneously with a worldwide incidence of 1-2 per million of population per year. Increased expression of PrP^c in relevant cellular compartments of the body in elderly people may facilitate the development of sporadic CJD, similarly to the hypersensitivity to prions of *tga20* mice, which overexpress wild-type PrP^c.[44] It will therefore be interesting, in further studies, to examine age-related PrPc expression in other body tissues, including central and peripheral nervous system.

Monocytes only showed a trend towards higher surface PrP^C expression in the elderly compared to the dramatic increase observed in lymphocytes. Because monocytes expressed less surface PrP^c per unit area than lymphocytes in all age groups, it is possible that differential expression on monocytes may

be related to the physiologic function of PrP^c: it has been claimed that PrP^C is important for activation of T-cells[11] although these results await independent confirmation.

Interestingly, expression of amyloid precursor protein, which is crucial in Alzheimer's disease, has also been reported to increase in lymphocytes with age.[45] Age-related increase in expression of proteins implicated in neurodegenerative pathologies could therefore be a more general phenomenon than previously thought.

What is different about vCJD and why it has so far affected only younger people remain an open questions. In the present study, surface PrP^c immunoreactivity was highly variable between different individuals in all age groups (Table 2): this variability proved true with all antibodies used. This variation may contribute to individual susceptibility to prion diseases, with strong overexpressors being more susceptible to disease.

Because the function of PrP^c is still largely unknown, we can only speculate on the reasons for the differences in PrP^C expression in various leukocyte subpopulations. A role for PrP^c in copper metabolism has been suggested based on the ability of the N-terminal octapeptide repeats of the protein to bind copper in vivo. A corresponding reduction in the copper content of brain membrane preparations has been documented in PrP deficient mice.[9] Availability of copper decreases with age:[46,47] therefore, upregulation of PrP^c in elderly PBM^C may help compensate for age-related copper deficiency. Copper is essential for the function of many proteins including transcription factors and proteins involved in oxidative damage prevention such as superoxide dismutase.[48] PrP deficient mice exhibit reduced superoxide dismutase activity and altered electrophysiologic responses in the presence of excess copper[9] and cerebellar PrP null mouse cells were more sensitive to oxidative damage compared to wild-type controls.[49] Copper deficiency has adverse effects on nervous and immune system function.[50-53] Conversely, excess copper is associated with cellular toxicity.[54] The fine regulation of copper is therefore very important for cell homeostasis.

T-lymphocyte function is more sensitive than B-lymphocyte function to copper deficiency and IL-2 mediated proliferation of T-lymphocytes in response to mitogens is reduced with low copper diet.[55-57] Thus our finding that human T lymphocytes expressed significantly more PrP^c than B-lymphocytes, may be related to a higher requirement of these cells for copper. In this respect it is of note that PrP^c has been previously shown to participate in murine T-lymphocyte activation and PrP null mice exhibit impaired responses to the mitogen ConA.[11] The precise role of PrP^C-bound copper awaits further investigation. PrP^c may have a protective role, shuttling copper ions to enzymes that prevent oxidative damage. Higher levels of PrP^c expression in long-lived cells would be consistent with a protective function for copper binding PrP^c. Neurons have indeed the highest level of surface PrP^c expression. We found higher surface PrP^c in the PBMC of elderly people, which have a higher proportion of long-lived memory lymphocytes.[58] CD8$^+$ T- lymphocytes had significantly higher levels of surface PrPc than CD4$^+$ T-lymphocytes. CD8$^+$ T-lymphocytes may need higher protection against oxidative damage during their own cytotoxic activities and could express higher levels of molecules such as PrP^c involved in copper metabolism, for that reason.

Further studies will aim to explore age-dependent PrP^c expression on all peripheral lymphoid tissues, including follicular dendritic cells in the lymph nodes. Since the immune system plays an important role in the propagation of spongiform encephalopathies, it may provide a target for interference with the development of these diseases and a system to study the physiological role of PrP^c further.

Potential implications for clinical practice

- Victims of new-variant Creutzfeldt-Jakob disease (nvCJD) are unusually young. Age-dependent variability in cell surface PrP expression is a candidate susceptibility factor.
- expression of PrP on various subclasses of human peripheral blood leukocytes (PBL) was shown to increase in age. Therefore, expression levels of PrP in blood cells are highly unlikely to contribute to nvCJD susceptibility.
- because the elderly expresses the highest amounts of PrP on PBL, variability in cell surface expression of PrP in PBL may contribute to the development of sporadic CJD in elderly individuals.

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GP performed the experiments described here with the help of JDS, and under the supervision of AA. MS provided the samples. The idea for this study was borne out of discussions between AA, HPS and GP. We wish to thank Dr. P. Stahel in the Department of Surgery, University Hospital of Zürich, for providing us with elderly patient's blood, Dr. B. Seifert in the Department of Biostatistics of the University of Zürich for his help with statistical analysis, and Mrs E. Niederer (ETH, Zürich) for technical assistance with FACS. The monoclonal antibody 3F4 was a kind gift from Professor R.J. Kascsak.

The order of authors is a joint decision of all authors and is based on the amount of work spent on executing (left to right) and supervising (right to left) the experiments described in this study.

Disclosures

Conflict of interest: none

Redundant publications: no substantial overlapping with previous papers.

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