Cerebrospinal fluid soluble L-selectin (sCD62L) in meningoencephalitis

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Abstract

The leucocyte adhesion molecule L-selectin (CD62L) is rapidly cleaved off proteolytically after cell activation, generating soluble L-selectin (sCD62L) molecules. sCD62L concentrations were determined in 185 cerebrospinal fluid (CSF) samples obtained from children aged 1 month to 17 years. In 36 CSF samples of children with meningoencephalitis, sCD62L was significantly higher (median 209 fmol/ml) than in samples of children with other febrile diseases (n=67, median 50 fmol/ml) or non-febrile disorders (n=82, median 44 fmol/ml). There was a positive correlation between CSF protein and CSF sCD62L ($r_s=0.68$), suggesting that a disturbed blood-brain barrier contributes to raised sCD62L concentrations in the CSF. However, the CSF sCD62L/protein ratio of children with meningoencephalitis was significantly higher than in children with other febrile diseases or non-febrile disorders, indicating that sCD62L concentrations in children with meningoencephalitis were higher than expected from plasma leakage alone. It is concluded that both an impaired blood-brain barrier and the generation of sCD62L by infiltrating leucocytes contribute to raised CSF sCD62L concentrations in children with meningoencephalitis.

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Keywords: cerebrospinal fluid, soluble L-selectin, meningoencephalitis.

The leucocyte surface adhesion glycoprotein L-selectin (CD62L) mediates reversible attachment of flowing leucocytes to altered or specialised endothelium¹ and endothelium bound leucocytes² which precedes leucocyte extravasation. Following cell activation, CD62L is rapidly cleaved off,^{3,4} and shed soluble L-selectin (sCD62L) appears in the surrounding fluid as 62 kDa and 75–100 kDa fragments with preserved ligand binding capacity.⁵ It has been reported that plasma or serum sCD62L concentrations were increased in patients with insulin dependent diabetes mellitus,⁶ acquired immunodeficiency syndrome,⁷ and a proportion of patients with acute leukaemia,⁸ while decreased sCD62L was found in premature newborn infants^{9,10} and neutropenic patients after bone marrow transplantation.¹¹ Low concentrations of sCD62L have also been detected in cerebrospinal fluid (CSF), with marked elevations in patients with meningeal leukaemia.¹² The aim of this study was to

determine whether sCD62L concentrations were increased in the CSF in children with acute inflammatory central nervous system (CNS) diseases.

Material

PATIENTS

sCD62L concentrations were measured in 185 CSF leftovers of samples submitted to the routine paediatric laboratory between January 1993 and August 1994. The analysis was restricted to children between 1 month (corrected for prematurity) and 17 years of age without haemorrhagic or neoplastic CNS diseases. Blood contaminated CSF samples (>30 red blood cells/ μ l) were excluded.

Samples were retrospectively allocated to three diagnostic categories: diagnostic lumbar puncture during sepsis work-up or after a febrile seizure, without a subsequent diagnosis of inflammatory CNS disease (n=67); non-febrile, non-pleocytotic disorders, such as Bell's palsy, mental retardation, or suspected metabolic disease (n=82); and acute inflammatory CNS diseases (meningitis, encephalitis, or meningoencephalitis, henceforth referred to as meningoencephalitis, n=36). Diagnostic criteria for the last group were fever, clinical signs of meningeal irritation or increased intracranial pressure, and CSF pleocytosis (>8 nucleated cells/ μ l). Ten of these 36 samples were from cases with bacterial aetiology, while in the other 26 samples meningoencephalitis was presumed to have resulted from viral infection.

The average age was lower ($p<0.05$) in the group of febrile children without CNS inflammation [median (10–90th centile range): 24 (3–122 months)] than in the groups of children with meningoencephalitis [67 (3–122 months)] and non-febrile disorders [62 (3–177 months)], while the latter two groups did not differ significantly with respect to age ($p>0.1$).

MEASUREMENTS

Centrifuged CSF samples were stored frozen at -20°C and analysed within 3 months. Immunoreactive sCD62L has been shown to be exceptionally stable during prolonged storage and after repeated freezing and thawing.⁵ CD62L concentrations were determined by a luminescence based sandwich ELISA as described previously,⁹ with an assay sensitivity of 17 fmol/ml (negative control +3 SD). Samples were diluted with an equal amount of buffer, or more if the first measurement yielded an sCD62L concentration above the upper end of the linear range of the assay

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Figure 1 Cerebrospinal fluid protein content (X axis) and sCD62L concentrations (Y axis) in children with acute meningoencephalitis, other febrile diseases, and non-febrile, non-pleocytotic disorders.

(100 fmol/ml). CSF protein content was determined by the biuret method.

STATISTICAL ANALYSIS

Non-parametric statistical tests with two sided *p* values were used throughout and were based on a significance level of 0.05. Results of groups are expressed as median and 10–90th centile range. Differences between groups were assessed by the Wilcoxon or Kruskal-Wallis test. The Kolmogorov-Smirnov goodness of fit test was used to assess normal (Gaussian) distribution. Spearman rank order correlation coefficients (r_s) were calculated to measure the strength of association between numerical variables. Sensitivity, specificity, and positive and negative predictive values were calculated for different cut off points, and the area under a receiver operating characteristic (ROC) curve which plots sensitivity versus 1 – specificity was used to estimate diagnostic accuracy.¹³

Results

sCD62L IN CSF

In CSF samples of febrile children who underwent a diagnostic lumbar puncture but were not found to have signs of inflammatory CNS disease, sCD62L concentrations ranged from <17 fmol/ml (detection limit of the assay) to 194 fmol/ml, with a median of 50 fmol/ml and a 10–90th centile range of 17 to 84 fmol/ml (fig 1). The distribution of sCD62L concentrations did not differ significantly from a normal distribution ($p > 0.1$). CSF sCD62L concentrations in children without acute febrile

disease [median (10–90th centile range): 44 (17–119 fmol/ml)] lacked a normal distribution ($p < 0.0001$) but did not differ significantly from those of children with febrile disorders without CNS inflammation ($p > 0.1$). In contrast, CSF samples of children with meningoencephalitis [median (10–90 percentile range): 209 (62–888 fmol/ml)] had significantly higher sCD62L concentrations ($p < 0.0001$, for comparisons to both control groups), with a distribution differing significantly from normal ($p < 0.01$).

In samples of children with meningoencephalitis, there was a positive correlation between CSF nucleated cell counts and sCD62L levels ($r_s = 0.61$, $p < 0.001$). sCD62L concentrations were significantly ($p < 0.0001$) higher in CSF samples of meningoencephalitis of bacterial origin [685 (257–482 fmol/ml)] than in viral meningoencephalitis [143 (49–331 fmol/ml)], as were nucleated cell counts [2700 (49–7756) cells/l *v* 110 (15–780) cells/ μ l].

There was no significant association between the child's age at the time of lumbar puncture and sCD62L concentrations in the whole study group or in the two subgroups of children without pleocytosis. In children with meningoencephalitis, CSF sCD62L concentrations (but not nucleated cell counts) appeared to decrease with chronological age ($r_s = -0.51$, $p < 0.01$).

CSF PROTEIN CONTENT AND sCD62L/PROTEIN RATIO

The CSF protein content was significantly higher ($p < 0.0001$) in children with meningoencephalitis (median 490 mg/l, 10–90th centile range 250–2088 mg/l) than in children with non-febrile disorders [250 (150–614) mg/l] and children with other febrile diseases [250 (148–362) mg/l]. A significant association ($p < 0.001$) was found between CSF protein content and sCD62L concentrations within the total study population ($r_s = 0.68$) and within the three diagnostic groups (meningoencephalitis: $r_s = 0.80$; other febrile diseases: $r_s = 0.56$, non-febrile disorders: $r_s = 0.54$). However, the CSF sCD62L/protein ratio was higher ($p < 0.0001$) in children with meningoencephalitis [0.385 (0.171–0.902)] than in children with other febrile diseases [0.212 (0.098–0.348)] and children with non-febrile disorders [0.166 (0.064–0.290)]. The CSF protein content was higher ($p < 0.0001$) in samples from children with bacterial meningoencephalitis [1626 (519–4464) mg/l] than in children with viral meningoencephalitis [380 (232–799) mg/l], while the CSF sCD62L/protein ratio did not differ significantly ($p > 0.1$) between the two groups [bacterial: 0.565 (0.156–1.405); viral: 0.366 (0.152–0.677)].

CSF sCD62L AND sCD62L/PROTEIN RATIO AS DIAGNOSTIC INDICES

In CSF of children with acute febrile diseases (with and without CNS inflammation), an sCD62L cut-off value of 98 fmol/ml maximised the sum of sensitivity plus specificity for

Figure 2 Receiver operator characteristic curves of cerebrospinal fluid sCD62L concentrations, protein concentrations, and sCD62L/protein ratio plotting sensitivity versus 1-specificity to discriminate between febrile children with and without meningoencephalitis.

the diagnosis of acute CNS inflammatory disease. Thirty of 36 children with meningoencephalitis had sCD62 concentrations of >98 fmol/ml (sensitivity 0.83); of 33 CSF samples with sCD62L concentrations of >98 fmol/ml, 30 were from children with meningoencephalitis (positive predictive value 0.91). Sixty four of 67 febrile children without CNS inflammation had sCD62 concentrations of <98 fmol/ml (specificity 0.96); of 70 CSF samples with sCD62L concentrations of <98 fmol/ml, 64 were from children without CNS inflammation (negative predictive value 0.91). The maximised cut-off value of the sCD62L/protein ratio was 0.333, yielding a sensitivity of 0.67, a specificity of 0.85, and positive and negative predictive values of 0.71 and 0.83, respectively. The area under the ROC curve (fig 2) was 0.92 for sCD62L, and 0.79 for the sCD62L/protein ratio. For comparison, the area under the ROC curve was 0.86 for the CSF protein level.

When all CSF samples analysed were taken into account, the sCD62L cut-off value maximising the sum of sensitivity plus specificity for the diagnosis of acute CNS inflammatory disease was 90 fmol/ml. Sensitivity was 0.86, specificity was 0.88, and positive and negative predictive values were 0.63 and 0.96, respectively. The maximising sCD62L/protein ratio cut-off value 0.333 was associated with a sensitivity of 0.67, a specificity of 0.90, and positive and negative predictive values of 0.62 and 0.92, respectively. The area under the ROC curve was 0.91 for sCD62L, and 0.83 for the sCD62L/protein ratio.

CSF nucleated cell counts, sCD62L concentrations, and protein content had a similar diagnostic accuracy in discriminating between bacterial and viral meningoencephalitis, while the sCD62L/protein ratio was not helpful in distinguishing between the two aetiologies. The area under the ROC curve was 0.88 for the CSF nucleated cell count, 0.95 for the CSF protein content, and 0.96 for CSF sCD62L levels. The cut-off points of 400 nucleated cells/l, 0.690 mg protein/l, and 316 fmol

sCD62L/ml were each associated with a sensitivity of 0.90, a specificity of 0.89, a positive predictive value of 0.75, and a negative predictive value of 0.96.

Discussion

Under normal conditions, the bulk of CSF proteins derives from ultrafiltration of plasma across the blood-brain barrier. While no reference range for plasma sCD62L concentrations in children has been published yet, sCD62L values have been found to be 12.4 (SD 2.1) pmol/ml in adult cubital vein plasma, and 8.5 (2.2) pmol/ml in umbilical cord vein plasma⁹ employing the same assay as that used here to determine CSF sCD62L concentrations. In children with febrile diseases without evidence of inflammatory CNS disease, presumed to have neither a disturbance of the blood-brain barrier nor production of sCD62L within the CNS, the estimated sCD62L plasma/CSF ratio (approximately 170–250:1) appears to be similar to that of most other proteins.¹⁴ While a simultaneous determination of sCD62L in both plasma and CSF in the same patient to specifically address this question was not performed, the correlation between CSF sCD62L concentrations and CSF protein content suggests that injury to the blood-brain barrier increases CSF sCD62L concentrations alongside CSF protein concentrations.

In addition to reaching the CSF by ultrafiltration, some proteins are also produced within the CNS, such as immunoglobulins, β_2 microglobulin, transthyretin (prealbumin), transferrin, and inflammatory cytokines.^{15–17} The investigation presented here suggests that during acute inflammatory CNS diseases, sCD62L is also actively released by cells within the CNS, since the increase in CSF sCD62L markedly exceeds that of CSF protein. Leucocytes are the only cell type known to express CD62L, and infiltrating leucocytes are the most likely source of locally produced sCD62L. After activation both in vitro and in vivo, leucocytes of lymphoid and myeloid origin alike are characterised by loss of their of surface expressed CD62L.^{3 4 18–20}

Although a positive correlation was found between CSF nucleated cell counts and CSF sCD62L levels, the leucocytes suspended in the CSF at the time of lumbar puncture can be expected to contribute only a minor fraction of CSF sCD62L. Assuming there are approximately 30 000 CD62L molecules per unstimulated leucocyte,²¹ even complete shedding of CD62L within the CSF compartment would result in no more than 5×10^{-5} fmol sCD62L per cell. The upper limit of the proportion of sCD62L shed from leucocytes in the CSF sample can be calculated to be 3.8% (10–90th centile range 1.1%–49.3%). As a highly glycosylated glycoprotein, sCD62L is easily soluble in aqueous solutions; thus both leucocytes suspended in CSF and an unknown number of leucocytes attached to neuronal and glial structures may contribute to CSF sCD62L. Since it is well documented that shedding of sCD62L from leucocytes is largely

accelerated upon stimulation,^{3 4} sCD62L production reflects the number of infiltrating leucocytes as well as their state of activation. In addition, CSF sCD62L concentrations may also be influenced by the duration of the inflammatory process. Owing to the lack of data regarding the half life of sCD62L in CSF (at 37°C), accumulation of sCD62L produced by repetitive waves of infiltrating leucocytes in the CSF over time remains a matter of speculation.

The predominant infiltrating leucocytes in acute meningoencephalitis of bacterial and viral origin, neutrophils and lymphocytes respectively, require different signals for down-regulation of CD62L surface expression in vitro. Neutrophils shed their surface expressed CD62L molecules within minutes after contact with soluble mediators of inflammation, such as interleukin-1 or tumour necrosis factor- α ³ which have been shown to be present in CSF during acute bacterial meningoencephalitis.^{17 22 23} In contrast, lymphocyte CD62L shedding does not occur in response to the above mentioned cytokines or interferon- γ , appears to require prolonged antigenic stimulation, and may take several hours to be complete.²⁴ Thus both the distinct signalling cascades involved and the disparate number of infiltrating leucocytes may contribute to the difference in CSF sCD62L concentrations observed in bacterial and viral meningoencephalitis.

The results in children with febrile diseases without CNS inflammation may be used to define a reference value of sCD62L concentrations in the CSF. However, none of the assays currently employed to quantitate sCD62L in biological fluids appears to have been sufficiently standardised by recombinant or highly purified natural sCD62L, and there is some discrepancy as to the normal sCD62L serum range.^{5 7-10} Some investigators prefer to express their data as relative units.^{6 11} While conclusions drawn from measurements with the same assay system are not affected by this problem, definitive reference values will have to await comparative testing of recombinant or highly purified natural sCD62L. In addition, sCD62L found in human body fluids is actually composed of various molecular species with molecular weights ranging from 62 to 100 kDa,⁵ most probably because of variable glycation of the protein core. Since the monoclonal antibodies used in several ELISA systems do not discriminate between the various sCD62L isoforms generated by differential glycation, the common practice of conversion of ELISA generated data from fmol/ml to ng/ml is inaccurate (100 fmol/ml may correspond to anything between 6.2 and 10 ng/ml).

The area under the ROC curves for CSF sCD62L and the CSF sCD62L/protein ratio shows that both variables are fairly accurate in distinguishing children with and without meningoencephalitis. In addition, CSF sCD62L levels but not the CSF sCD62L/protein ratio discriminates between bacterial and viral meningitis. However, the sandwich ELISA used in this study takes four to five

hours, and necessary treatment would have to be instituted well before that time. This drawback is shared by investigations reporting raised CSF concentrations of other proteins in meningoencephalitis of bacterial or viral origin, such as β_2 microglobulin, tumour necrosis factor α , interleukin-1 β , interleukin-6, interferon- γ , or granulocyte colony stimulating factor.^{15 22 23 25} To establish the presence or absence of biologically significant changes, virtually all studies including this one referred to cases of acute meningoencephalitis. The results should encourage further investigation into subacute, chronic, or relapsing forms of inflammatory CNS disease, where additional measurements might not only provide useful ancillary diagnostic tools but also help to gain further insight into the mechanisms of disease.

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Multiple sclerosis

The Grampian region of Scotland has the highest recorded incidence of multiple sclerosis in the world. A register in Aberdeen includes 1489 cases and 28 of these had an onset of the disease before the age of 16 years. Data have recently been presented about these 28 patients (G F Cole and C A Stuart, *Developmental Medicine and Child Neurology* 1995; 37: 661-6).

Seven children had their first symptoms before the age of 10 and 21 between ages 10 and 15 years. Two were aged 1 and 2 years but the rest were 6 or over. The features of the disease were similar to those seen in adults but 11 children had a systemic illness at the onset which is uncommon in adults. Presenting features were: limb, bulbar or facial weakness (15), visual blurring (10), sensory loss (9), ataxia (3), bladder dysfunction (3), vertigo (2), and encephalopathy (2). A second episode occurred within a year in 17 patients but the longest interval was 25 years. Useful investigations were those used in adults: magnetic resonance imaging, visual evoked responses, and cerebrospinal fluid examination for IgG and oligoclonal bands.

On the whole the prognosis for children seemed somewhat better than for adults. Fifteen patients were well after follow up for between three and over 30 years. Eight died from three to 43 years after the onset. Four patients had a steadily progressive course from the onset. Twenty one had a relapsing-remitting course all along and two developed a progressive illness after a period of relapsing-remitting. One was lost to follow up.

One child aged 13 months at onset had a progressive course and died aged 9 and another aged 2 years at onset had a relapsing-remitting course and was well at the age of 15 years (G F Cole and colleagues, *Developmental Medicine and Child Neurology* 1995; 37: 667-72).

Multiple sclerosis may occur at any age past infancy and paediatricians should be prepared to consider the diagnosis, perhaps before the classical 'dissemination of lesions in space and time' has occurred.

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