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# sICAM-1 INTRATHECAL SYNTHESIS AND RELEASE DURING THE ACUTE PHASE IN CHILDREN SUFFERING FROM COXSACKIE A9 AND *S. pneumoniae* MENINGOENCEPHALITIS

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**Abstract** – The intercellular adhesion molecule is a transmembrane glycoprotein belonging to the immunoglobulin superfamily. Serum and cerebrospinal fluid (CSF) soluble intercellular adhesion molecule 1 (sICAM-1) from normal control children as well as from children with Guillain-Barré syndrome (GBS), with Coxsackie A9 virus meningoencephalitis and with *Streptococcus pneumoniae* meningoencephalitis were studied. sICAM-1 was quantified using an immunoenzimatic assay and albumin using the immunodiffusion technique in both biological fluids. Increased sICAM-1 values in CSF in patients with GBS correspond to an increase of the albumin CSF/serum quotient. In contrast, in inflammatory diseases like *S. pneumoniae* and Coxsackie A9 virus meningoencephalitis an increased brain-derived fraction was observed. In particular cases these values are 60-65% and 70-75% respectively. The results indicate an additional synthesis of sICAM-1 in subarachnoidal space during central nervous system (CNS) inflammatory process. An important role of sICAM-1 in the transmigration of different cell types into CSF during CNS inflammation in children with *S. pneumoniae* and Coxsackie A9 meningoencephalitis may be suggested.

KEY WORDS: cerebrospinal fluid, sICAM-1, Coxsackie A9, *Streptococcus pneumoniae,* children.

**Síntesis intratecal de sICAM-1 y liberación durante la fase aguda en niños con meningoencefalitis por Coxsackie A9 y *S. pneumoniae***

**Resumen** – La molécula de adhesión intercelular es una glicoproteína que pertenece a la superfamilia de las inmunoglobulinas. Se estudiaron los niveles de molécula de adhesión intercelular tipo 1 soluble (sICAM-1) en suero y líquido cefalorraquídeo (LCR) de niños con meningoencefalitis por *Streptococcus pneumoniae* y por Coxsackie A9 al igual que en niños con sindrome de Guillain-Barré (SGB). sICAM-1 fue cuantificado por ensayo inmunoenzimático y la albúmina por inmunodifusión en ambos líquidos biológicos. Los valores incrementados de sICAM-1 en LCR en los pacientes con GBS corresponden a valores aumentados de razón LCR/suero de albúmina. En contraste, en las enfermedades inflamatorias como las meningoencefalitis por *S. pneumoniae* y por Coxsackie A9 se observa un incremento en la fracción derivada del cerebro. En casos particulares los valores se incrementan hasta un 60-65% y 70-75% respectivamente. Los resultados indican una síntesis adicional de sICAM-1 en el espacio subaracnoideo durante el proceso inflamatorio del sistema nervioso central (SNC). Esto puede sugerir un importante papel del sICAM-1 en la transmigración de diferentes tipos celulares en el LCR durante la inflamación del SNC en niños con meningoencefalitis por *S pneumoniae* y coxsackie A9.

PALABRAS-CLAVE: líquido cefalorraquídeo, sICAM-1, Coxsackie A9, *Streptococcus pneumoniae,* niños.

Lymphocyte recruitment to the central nervous system (CNS) is a critical step in the pathogenesis of meningitis. The principle sequential stages that control lymphocyte emigration from the blood have been widely reported, but only recently has attention been directed towards the role of the vascular endothelium in actively supporting

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transvascular migration. It has now been shown that adhesion molecules, particularly those of the immunoglobulin super family like ICAM-1, not only act as ligands for leukocyte receptors but can also serve as signal transducers[1]. Intercellular adhesion molecule 1 (ICAM-1) is a transmembrane glycoprotein belonging to a immunoglobulin superfamily. Its normal status is to be present on many haematopoietic and non-haematopoetic cells including cells of the CNS. During inflammation and allergic reactions its expression is up-regulated, and it plays a crucial role in the transmigration of cells to the inflammation place.

ICAM-1, a cell surface receptor important for cellular interactions in immune responses, especially leukocyte trafficking into inflammed tissue, is released in a soluble form (sICAM-1) into the extracellular space. It is found in many body fluids, including cerebrospinal fluid (CSF). About 60% to 80% of sICAM-1 in normal lumbar CSF derives from blood. This calculation is based on the theoretically expected molecular size-dependent blood-CSF gradient between 300:1 to 250:1[2].

The aim of this study is to evaluate the possible participation of sICAM-1 in the immune response of CSF in children suffering from Coxsackie A9 and *Streptococcus pneumoniae* (*S. pneumoniae*) meningoencephalitis, reported for first time.

## METHOD

### Patients

Eleven children suffering from Coxsackie A9 meningoencephalitiis and ten children with *S. pneumoniae* meningocencephalitis with ages between 3 and 10 years and a control group were studied. This later group was formed of nine children without any organic brain disorder and three patients with Guillain-Barre syndrome (GBS). Lumbar puncture was performed on the day of admission of children with symptoms of eosinophilic meningoencephalitis.

### Routine CSF/serum analysis

Routine CSF/serum analysis was performed in all cases according to the protocol described earlier[3]. CSF samples contaminated with blood were not included in the study. For measurements of sICAM-1, aliquots of CSF and serum were frozen and kept at –20ºC for further analysis in groups of 10–12 samples.

sICAM-1 in CSF and serum were analyzed using sandwich ELISA methods (R&D Systems Europe, UK). The sensitivity of the ELISA was determinate by a serial dilution of the standards in the sample diluents (both included in the kit). The lowest concentration distinguishable from the blank was 0.35 ng/mL. Thus, the assay was found sensitive enough to determine sICAM-1 concentration in all samples. Additionally, results obtained with two assays (R&D Systems Europe, UK, and Bender MedSystems Austria) were compared and found to be essentially equivalent. A positive control serum is included in the assays and was measured twice during each run. The day-by-day coefficient of variation (inter-assays imprecision) was 5.5%. Undiluted CSF samples were used for the assays and serum was diluted in a ratio of 1:20. All steps of the procedure followed the manufacturer's instructions. Absorbance was measured with an automatic ELISA reader (SLT Lab instruments, Germany) using an evaluation program (easy-fit) from the same manufacturer.

Serum and CSF albumin were quantified by radial immunodiffusion technique in NOR and LC Partigen immunoplates respectively (Dade-Behring, Marburg). The radial immunodifussion technique is a very useful one and it is the golden rule for protein quantification. It is advisable for quantifying small number of samples.

## RESULTS

### Routine CSF/serum analysis

All cases of Coxsackie A9 and *S. pneumoniae* meningonencephalitis showed typical results on the routine CSF/serum analysis[4-9]; increased CSF total protein with increased Q albumin in the majority of the cases with bac-

*Table. Data obtained in control group and in different neurological diseases.*

| Group | n | sICAM-1 mean (SD) | | Q alb x $10^{-3}$ | p | QsICAM-1 x $10^{-3}$ | p |
|---|---|---|---|---|---|---|---|
| | | SERUM ng/mL | CSF ng/mL | mean (SD) | | mean (SD) | |
| Controls[1] | 9 | 338.4 (145.7) | 1.25 (0.21) | 4.9 (2.28) | | 4.81 (3.20) | |
| GBS | 3 | 287.7 (45.7) | 3.4 (1.81) | 15.93 (10.06) | 0.007 S | 15.80 (13.99) | 0.037 S |
| Cox A9 meningoencephalitis | 11 | 298.8 (67.89) | 2.53 (2.21) | 4.58 (1.94) | NS | 11.81 (8.82) | 0.035 S |
| *S. pneumoniae* meningoencephalitis | 10 | 437.0 (274.0) | 9.54 (3.1) | 12.68 (8.92) | 0.021 S | 24.91 (22.90) | 0.018 S |

[1]Children with non-inflammatory CNS diseases, without other organs involved; Children who underwent to Lumbar puncture due to suspected neurological inflammatory disease; CSF/serum analysis as well as follow-up observation in Hospital excluded CNS inflammation, confirming inflammation of other organs like pneumonia, diarrhoea, etc. Children probably punctured before steady-state conditions; S, significant differences; NS, not significant differences.

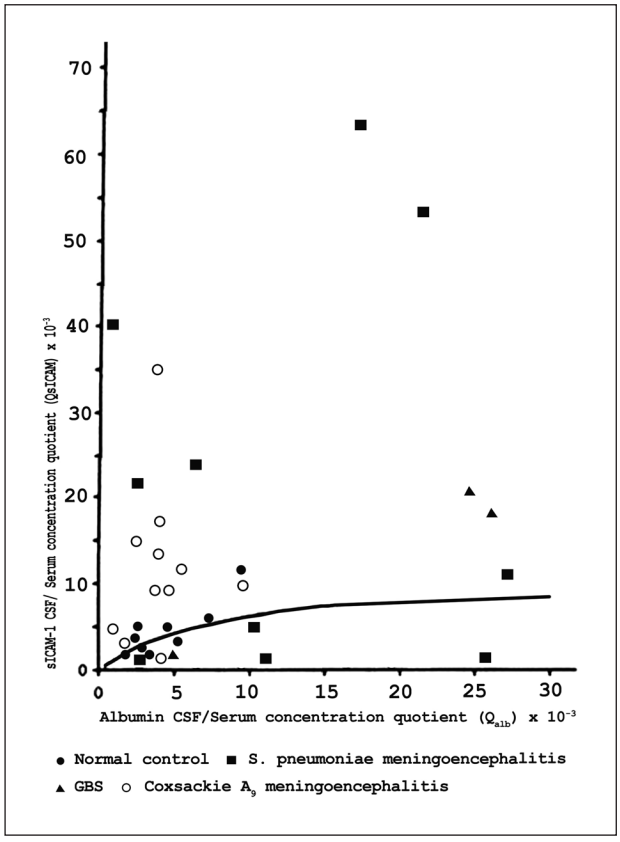


*Figure. Relationship R(sICAM) and $Q_{alb}$.*

terial meningoencephalitis, and in some patients suffering from viral meningoencephalitis. Intrathecal immunoglobulin responses were found in the first diagnosis lumbar puncture as a typical fact in children as is our experience[10-15].

**sICAM-1 in CSF/serum: sICAM-1 quotient $Q(s_{ICAM})$ and $Q_{Alb}$**

Table shows sICAM and albumin values and its Q values. Albumin and sICAM-1 values obtained were presented as CSF/serum quotients (Q Albumin and Q sICAM-1). Normal distribution of Q Albumin and Q sICAM-1 for the different groups were obtained using the Kolmogorov-Smirnov test.

Q albumin is a marker of the blood-CSF dysfunction. Mean Q albumin in GBS and in *S. pneumoniae* shown significative differences as compared with controls.

Mean Q sICAM-1 was significant different in GBS and in *S. pneumoniae* and in Coxsackie A 9 meningoencephalitis from the control values.

Figure shows the relationship between $Q(s_{ICAM})$ and $Q_{alb}$ for all the pediatric patients with viral and bacterial meningoencephalitis. The curve was performed using the controls described previously paper[2,3]. The curve is based on data obtained from children and adults with control neurological diseases including patients with GBS. The curve represents the upper range of the values obtained from these control diseases[2].

Two patients with GBS were not located on the curve. It is possible because the curve is not a disease-specific one despite the curve was based on data including a group of patients with this disease.

Ten patients with Coxsackie A9 and six with S. pneumoniae were above the discriminatory curve that separates patients with a release of sICAM, as reported previously.

The patients from the control group exhibit no sICAM-1 values over the discrimination line, i.e, no increased brain-derived fraction.

When sICAM-1 is not intrathecally produced, i.e., only the brain-derived fraction is present and 30-40% of its content is in serum.

Increased values in CSF in patients suffering from GBS correspond to an increase of Q albumin. In contrast, in inflammatory diseases like *S. pneumoniae* and Coxsackie A9 meningoencephalitis an increased brain-derived fraction was observed. In particular cases these values are up to 60-65% and 70-75% respectively.

## DISCUSSION

A hallmark of infectious meningitis is the invasion of leukocytes into the subarachnoid space. ICAM-1-mediated signalling in brain endothelial cells is a crucial regulatory step in the process of lymphocyte migration through the blood-brain barrier and as such represents an additional phase in the multistep paradigm of leukocyte recruitment.

Earlier published reports on sICAM-1 have been controversial due to less sensitive assays and unsuitable linear evaluation concepts for blood-CSF barrier dysfunction but later this problem was solved and several papers have confirmed the non-linear distribution of this protein according to its albumin quotients[2,3].

Other authors confirm that the correlation between CSF and serum concentrations of adhesion molecules and the presence of a discrepancy of CSF/serum ratios for molecules of the same molecular weight may suggest intrathecal shedding in addition to diffusion through blood-CSF barrier[16].

Adhesion molecules play a key role in leukocyte migration into the CNS. Data from studies of endothelial cells-related adhesion molecules, underscore the importance of the adhesion molecules in facilitating the movement of leukocytic and human pathogens across the blood-brain barrier during inflammatory and neoplasic events[17].

sICAM-1 may be an agent in the negative feedback for eosinophils passage through the blood-CSF barrier into the inflammated brain in eosinophilic meningitis due to *Angiostrongylus cantonensis*. It has been suggested that a

dynamic of the sICAM-1 brain derived fraction is perhaps associated to the immune response in the evolution of the disease[3].

The upregulation of adhesion molecules, ICAM-1, VCAM-1 and E-Selectin, in the pathogenesis of cerebral malaria (CM) are well established and were maximally upregulated in the cerebellar sections of the malaria cases[18].

The increased intrathecal release of sICAM-1 in viral meningoencephalitis and bacterial meningitis most likely reflects activation of macrophages and lymphocytes and provides evidence for a strong local immune response that itself, in addition to the infectious agent, may damage nervous tissue.

A recent paper studied the absolute values of sICAM-1 neuroborreliosis. The results of this study reconfirmed the participation of intercellular adhesion molecules in the pathogenesis of neuroborreliosis[19].

Meningitis caused by *S. pneumoniae*, the most common agent in adults in general and the most common in childhood in our country, is still associated with a surprisingly high mortality and 50% of the survivors suffer from neurological sequelae.

Depletion of the meningeal macrophages and perivascular macrophages during experimental pneumococcal meningitis resulted in increased illness, which is correlated with higher bacterial counts in the CSF and blood. This was associated with a decreased influx of leukocytes into the CSF, which occurred despite an elevated production of relevant chemokines (e.g., macrophage-inflammatory protein-2) and a higher expression of vascular adhesion molecules[20].

In *S. pneumoniae* meningoencephalitis we have observed sICAM-1 intrathecal synthesis and release into CSF in the majority of the studied cases. In a previous study with bacterial meningoencephalitis, the brain-derived fraction of sICAM-1 in CSF was up to 12-fold higher than that in controls[2].

In general the mean value of Q sICAM-1 has significant difference in comparison with the control group, but it is interesting that 5 of the *S. pneumoniae* meningoencephalitis cases are under the curve. The extremely large range variability in this disease group appears perhaps because there are patients in which process ICAM-1 could be released before the initial symptoms appear, when the diagnostic lumbar puncture was performed.

Analysis of adhesion molecule levels in other viral meningitis demonstrated indirect evidence of brain-derived fractions. For instance it has been suggested the involvement of adhesion molecules during the early phase of mumps meningitis[21] and other viral meningoencephalitis[22].

The brain perivascular compartments are highly relevant to pathologies affecting the CNS, as infections are almost exclusively blood-borne. Insults disrupt blood and energy flow to neurons, and active brain-to-blood transport mechanisms, which are the bottleneck in the clearance of unwanted molecules from the brain. Perivascular macrophages are the most reactive cell type and produce IL-1beta and TNFalpha after infection or injury to the CNS. The main cellular target for IL-1beta and TNFalpha produced in the brain perivascular compartment is the endothelium, where these cytokines induce the expression of adhesion molecules and promote leukocyte infiltration[23].

Exciting, ongoing clinical trials are addressing possible therapeutic intervention in neuroinflammatory diseases, including multiple sclerosis and meningoencephalitis, by blocking certain glycoprotein adhesion molecules before cells have the ability to adhere to the endothelial cells and migrate across the blood-brain barrier. Approaches whereby inflammation may be reduced or arrested using anti-adhesion molecules, by restructuring endothelial cell cytoskeletal, filamentous proteins, as well as remodelling cholesterol components are discussed in the context of developing future therapies for blood-brain barrier injury and inflammation. Understanding new concepts about the mechanism(s) by which inflammatory cells and a variety of pathogenic microorganisms are transported across the blood-brain barrier can be expected to advance our understanding of fundamental disease processes[17].

Our results indicate an additional synthesis of sICAM-1 in subarachnoidal space during CNS inflammatory process. They might suggest an important role of sICAM-1 in transmigration of different cell types into CSF during CNS inflammation in children with S. pneumoniae and Coxsackie virus meningoencephalitis.

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