Increased interleukin-6 levels in cerebrospinal fluid following subarachnoid hemorrhage

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Serum and cerebrospinal fluid (CSF) samples from 12 patients were analyzed for interleukin (IL)-6, soluble IL-2 receptor (IL-2R), and soluble CD8 levels in order to determine the immune activation profile following subarachnoid hemorrhage (SAH). Dramatically increased levels of IL-6 and moderate increases of soluble IL-2R were detected in the CSF in 11 of the 12 patients; slightly elevated levels of soluble CD8 were observed in six patients. The IL-6 levels were higher on Day 6 than on Days 3 and 9. The increases in IL-6, soluble IL-2R, and soluble CD8 levels in the CSF samples were not paralleled by increased values in the serum samples, and thus probably reflected an intrathecal synthesis of the cytokine. Passive transfer of IL-6 across the blood-brain barrier seemed not to occur since the serum and CSF levels of IL-6 showed a negative correlation. The findings suggest a severe inflammatory affection of the central nervous system that could be of importance in understanding the clinical course in patients following SAH.

KEY WORDS: subarachnoid hemorrhage, interleukin-6, interleukin-2 receptor, CD8, delayed ischemic deficit, inflammatory response

IMMUNOMODULATORS regulate cellular events within the immune system and may influence cellular events within the brain.4,9,16 The immune reactions in the brain may differ from those encountered systemically since the blood-brain barrier (BBB) obstructs a free diffusion of cells and molecules belonging to the immune system. Localized brain immune responses lead to increased levels of the immunomodulators "cytokines."

Subarachnoid hemorrhage (SAH) is frequently accompanied by an increased leukocyte count in peripheral blood and a low-grade fever, indicating a systemic inflammatory response.5,12 An inflammatory reaction in the central nervous system (CNS) seems likely based on previous studies.13,25,26 The T-cell activation marker neopterin was increased in the cerebrospinal fluid (CSF) following SAH.18 Cytokine production in the CNS following SAH and its relation to clinical outcome has not been studied. The present study was undertaken to analyze whether systemic and/or CNS levels of interleukin (IL)-6 would increase following SAH. Soluble IL-2 receptors (IL-2R) and soluble CD8 levels were measured in serum and CSF since their appearance would indicate the presence of activated T cells in the CNS and make it possible to determine whether such T cells would be mainly CD8-negative or CD8-positive.

In addition, an attempt was made to analyze IL-6 levels separately in patients suffering from delayed ischemic deficit.

Clinical Material and Methods

Patient Population

The clinical characteristics and outcome in 12 consecutive patients with SAH are summarized in Table 1. Of the 12 patients, 10 suffered from spontaneous SAH and two from traumatic SAH. An aneurysm was demonstrated in eight patients with spontaneous SAH. Delayed ischemic deficit, defined as neurological deterioration in the absence of hydrocephalus, rebleeding, and major electrolyte derangement, was detected in five patients. The deficit became permanent in one patient (Case 1).

Serum and Cerebrospinal Fluid Samples

A total of 31 CSF and 19 serum samples were collected from the 12 patients. The samples were immediately centrifuged and stored at −20°C until analysis. The CSF samples were obtained on Days 3 and 6 following ictus from nine patients with spontaneous SAH and one with traumatic SAH, and on Days 1 and 9 from four and five patients, respectively, with spon-
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<td>12</td>
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<td>50</td>
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* Abbreviations: SAH = subarachnoid hemorrhage; PCoA = posterior communicating artery; MCA = middle cerebral artery; ACoA = anterior communicating artery; PICA = posterior inferior cerebellar artery; - = absence of delayed ischemic deficit; + = temporary delayed ischemic deficit; ++ = permanent delayed ischemic deficit.

† Outcome: 5 = asymptomatic and independent; 4 = mild symptoms, independent; 3 = moderate deficit, dependent on some assistance; 2 = severe deficit, institutionalized; and 1 = dead.

Single CSF samples from one patient with spontaneous SAH and one with traumatic SAH were obtained on Days 3 and 6, respectively. Subarachnoid hemorrhage was diagnosed by computerized tomography or by analysis of CSF obtained via lumbar puncture. Samples were not obtained from patients who were surgically treated, subjected to steroid treatment, or suffering from septic conditions during the study period.

Serum and CSF samples were obtained from 18 to 22 adults without evidence of inflammatory diseases, nervous system trauma, or vascular disease, and these served as controls. Values above the mean ± 2 standard deviations for the control samples were classified as abnormal.

**Analysis of IL-6, Soluble IL-2R, and Soluble CD8 Levels**

The levels of IL-6 were analyzed with enzyme-linked immunosorbent assay (ELISA) reagents. * Incubation with serum, standards, and conjugate was performed according to the instructions of the manufacturer, using only half the volumes. Substrate activation by horseradish peroxidase was determined by chemoluminescence. † Tetramethylbenzidine (100 μl, 0.1 mg/liter) was added and the solution was incubated for 20 minutes. The plate was then placed in a luminometer and 50 μl of 6 mM Na Luminol, ‡ which had been recrystallized three times and stabilized with hydrogen peroxide, was added to each well. Emitted light was measured by means of a photomultiplier with a spectral response of 350 to 680 nm. The signal was measured at a peak over a total time period of 1 second. Light signals over background values were detected linearly related in a log scale in the 5.0- to 2000-ppg/ml interval of IL-6 in the standard curve. For the analysis of soluble IL-2R and soluble CD8 levels, commercially available ELISA kits were used. As with IL-6, the manufacturer's instructions were followed except for the last steps, when substrate activation was determined by chemoluminescence as described above.

**Results**

**Interleukin-6 Levels**

In the control samples, the mean values of IL-6 were 41 ± 13 pg/ml in CSF (18 samples) and 8 ± 8 pg/ml in serum (22 samples). Values below 67 pg/ml in CSF and 24 pg/ml in serum were regarded as normal.

Serum IL-6. The levels of IL-6 in serum were analyzed in eight patients (Fig. 1). On Day 3, the levels were normal in four patients (Cases 3, 9, 10, and 12) with spontaneous SAH and in one (Case 11) with traumatic SAH. An increase above normal values was observed in two patients (Cases 1 and 3) with spontaneous SAH and in one (Case 2) with traumatic SAH. An increase from normal on Days 3 and 6 to supranormal on Day 9 was detected in one patient (Case 11) with traumatic SAH.

Cerebrospinal Fluid. The levels of IL-6 in CSF were analyzed in all 12 patients (Fig. 1), and did not correlate with serum IL-6 levels (r = -0.45). Pathologically elevated values were detected on Day 1 and/or Day 3 in 11 patients; samples for Day 3 were not available from one patient (Case 2) with traumatic SAH. A marked increase in levels from Day 3 to Day 6 was observed in eight patients with spontaneous SAH. One patient (Case 3) died on Day 4 and thus could not be evaluated further. The values decreased from Day 3 to Day 6 and/or Day 9 in two patients (Cases 4 and 8). Peak IL-6 values were detected on Day 6 following SAH (Fig. 1). These peak values, around 300-fold higher than those in control samples, were observed in five patients. A statistically significant increase was detected from Day 3 to Day 6 in the patients with spontaneous SAH (p < 0.005, paired t-test). From Day 6 to Day 9, a decrease was detected in the six patients available for comparison (p < 0.05). A subgroup analysis of the 10 patients with spontaneous SAH was performed, showing a significant increase from Day 3 to Day 6 in the five patients with delayed ischemic deficits (p < 0.01) but not in the five patients without deficits (p > 0.10). The CSF IL-6 values on Day 3 showed a high correlation with the soluble IL-2R values in serum (r = 0.87) and CSF (r = 0.85). On Day 6, the correlation coefficient between CSF IL-6 and CSF soluble IL-2R was 0.77. The CSF IL-6 values did not correlate with serum soluble IL-2R on Day 6 or with serum or CSF soluble CD8 values at any time.

* ELISA reagent supplied by Quantikine Research and Diagnostic Systems, Minneapolis, Minnesota.
† Luminoscan manufactured by Labsystems, Inc., Tyresö, Sweden.
‡ Tetramethylbenzidine and Na Luminol supplied by Sigma Chemical Co., St. Louis, Missouri.
§ Cell-free soluble IL-2R and soluble CD8/T8 test kit manufactured by T-Cell Sciences, Inc., Cambridge, Massachusetts.
Soluble IL-2R Levels

In the control samples, the mean soluble IL-2R levels were 10 ± 5 U/ml in CSF (20 samples) and 570 ± 200 U/ml in serum (19 samples). Values below 20 U/ml in CSF and 970 U/ml in serum were regarded as normal.

Serum Soluble IL-2R. The levels of soluble IL-2R in serum were analyzed in eight patients, six with spontaneous SAH and two with traumatic SAH (Fig. 2). On Day 1 and/or Day 3, the levels were normal in five patients (Cases 1, 3, 8, 9, and 12) with spontaneous SAH and elevated in two (Cases 2 and 11) with traumatic SAH and in one (Case 10) with spontaneous SAH. Serum soluble IL-2R increased in supranormal values in one patient (Case 1) with spontaneous SAH, yielding a total of two of the six patients with spontaneous SAH who had supranormal serum soluble IL-2R levels on Day 6 and/or Day 9.

Cerebrospinal Fluid Soluble IL-2R. The levels of soluble IL-2R in CSF were analyzed in all 12 patients (Fig. 2), and did not correlate with the corresponding serum values. Moderately increased CSF values were detected on Day 1 and/or Day 3 in 11 patients. A further increase on Day 6 was observed in three patients (Cases 1, 5, and 9). One patient (Case 3) died on Day 4 and could not be evaluated further. The highest CSF soluble IL-2R values were thus detected by Day 3 in six of 10 patients with spontaneous SAH. Statistically significant changes were not observed between Days 1, 3, 6, and 9. The CSF soluble IL-2R values correlated with CSF IL-6 values but not with serum or CSF soluble CD8 values.
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Cerebrospinal Fluid Soluble CD8. The levels of soluble CD8 were analyzed in all 12 patients (Fig. 3). Moderately elevated values were detected on Day 1 and/or Day 3 in four patients (Cases 5, 6, 7, and 10). An increase from Day 3 to Day 6 was observed in four patients (Cases 1, 2, 4, and 7). One patient (Case 3) died on Day 4 and could not be evaluated further. The highest soluble CD8 values were detected by Day 3 in three patients (Cases 5, 6, and 10). As in IL-6, no distinct time kinetics was observed.

Delayed Ischemic Deficit

A delayed ischemic deficit was diagnosed in five patients (Cases 1, 6, 7, 9, and 12). The mean CSF IL-6 levels in these patients increased from 120 pg/ml on Day 3 to 1440 pg/ml on Day 6 (p < 0.05, paired t-test). The CSF soluble IL-2R levels increased in four of these patients from Day 1 and/or Day 3 to Day 6 and/or Day 9. The mean soluble IL-2R level on Day 6 was 56 U/ml.

No delayed ischemic deficit was detected in six of the 11 patients surviving through the study period. Four of these patients suffered spontaneous SAH; their CSF IL-6 levels changed from a mean of 410 pg/ml on Day 3 to 550 pg/ml on Day 6 (p > 0.10, paired t-test). Including the two patients with traumatic SAH, the mean CSF IL-6 levels for the six patients without delayed ischemic deficits increased from 290 pg/ml on Day 3 to 630 pg/ml on Day 6 (not statistically significant, p > 0.10). The mean CSF soluble IL-2R level on Day 6 was 60 U/ml. The CSF soluble IL-2R levels decreased in five of these six patients from Day 1 and/or Day 3 to Day 6 and/or Day 9.

Discussion

In the present study, increased levels of inflammatory markers and mediators were found in the CSF following SAH. The findings corroborate earlier reports that SAH causes an inflammatory reaction in the CNS. The present analysis of CNS inflammation following SAH revealed a marked increased, sometimes more than 300-fold, in CSF IL-6 above normal values. This increase was not paralleled by a systemic increase of IL-6 measured in serum and thus reflected an intrathecal synthesis or secretion of the cytokine. Passive transfer across the BBB seemed to be a negligible source of CSF IL-6 since the serum and CSF levels of IL-6 showed a negative correlation. An inflammatory reaction that is regulated differently in the CNS than in peripheral blood compartments has been described in other diseases of the nervous system. Two different pathways of IL-6 synthesis have been postulated in the CNS. The source of IL-6 may be the traditional cell of the immune system: T cells or monocytes. It is, however, intriguing that astrocytes and microglia are also capable of secreting IL-6. The immunological functions of brain cells are complex and have been studied only recently.

The present study revealed increased CSF soluble IL-2R levels early following SAH. They were increased between Days 1 and 3 in 10 of the 12 patients. The
absence of a correlation between serum and CSF concentrations of soluble IL-2R following spontaneous SAH suggests an intrathecal rather than a systemic source of CSF soluble IL-2R. Thus, the soluble IL-2R levels probably reflect the presence of activated T cells in the CNS, resulting from either the original SAH or leakage through the BBB at a later date. A correlation was detected between CSF IL-6 and soluble IL-2R concentrations in both CSF and serum. The activated T cells seem to have been present in the CNS and in peripheral blood simultaneously with the peak IL-6 values. A causal relationship may exist between the soluble IL-2R and IL-6 values, but it is also possible that they were produced independently by different cells and following different activation signals.

**Literature Review**

Experimental studies have revealed increased CNS levels of IL-1 and IL-6 following brain trauma in the rat. In humans, IL-6 is produced in the CNS in patients with viral CNS infections or CNS neoplasia, but not in patients with multiple sclerosis. The soluble IL-2R levels in CSF are markedly increased in patients with CNS infections, multiple sclerosis, or CNS neoplasm. The soluble IL-2R elevation detected in the SAH patients in this series was mild in comparison to the five-fold increase in CSF soluble IL-2R levels reported in multiple sclerosis patients, while the increase in IL-6 was dramatic. The CNS immune responses thus appear different in patients with spontaneous SAH and in those with multiple sclerosis or viral CNS infections. A difference is also indicated between viral infections on one side and bacterial infections, trauma, and SAH on the other. Viral infections mainly appear to produce T-cell activation markers (interferon-γ and soluble IL-2R) in the CNS, while products of monocytes and glial cells (IL-1, tumor necrosis factor-α, and IL-6) seem to be associated with the latter diseases.

In patients with viral CNS infections, soluble IL-2R levels tend to be higher in patients with acute infection, whereas postinfection neurological disorders seem to correlate with higher levels of soluble CD8, an indicator of activated CD8-positive cells commonly identified as the cytotoxic/suppressor lymphocyte subpopulation. Soluble CD8 concentrations in CSF are increased in immune-mediated CNS diseases caused by previous viral infection, including human immunodeficiency virus. In the present study, the mildly elevated CSF soluble CD8 levels following SAH in six of the 12 patients may have resulted from the original hemorrhage or may reflect a later event. Considering the absence of a marked soluble CD8 elevation, the majority of cells producing soluble IL-2R must have been CD8-negative. This would be compatible with the findings of Freedman, et al., that soluble CD8-positive cells appear to be excluded to some degree from the CSF.

In contrast to the CSF findings, serum levels of IL-6 and soluble IL-2R were not changed in a consistent pattern. Serum soluble CD8 was, however, increased at some time following SAH in all patients investigated. This finding suggests a consistent systemic activation of the suppressor/cytotoxic T-cell subpopulation and fits with previous reports of a systemic leukocytosis in patients with SAH.

The two patients with traumatic SAH showed comparatively higher serum levels of soluble IL-2R and soluble CD8 than those in the CSF. The highest serum levels of soluble IL-2R, soluble CD8, and IL-6, in the absence of marked CSF increases, were detected in these two trauma patients. Brain injuries are usually combined with trauma to other parts of the body as well. Thus, systemic immunoactivation following trauma may well explain this finding.

**Delayed Ischemic Deficit**

A pathogenic role of the immune system in vasospasm and delayed ischemic deficit after SAH has been postulated by some authors, while others have failed to detect its causative role. Different mechanisms of the immune system have been ascribed a role in delayed ischemic deficit. The intracerebral levels of IL-6 appeared to be increased on Days 1 through 3, then decreased, and eventually reached a maximum around Day 6 following ictus. The delayed ischemic deficit, most commonly noticed at 4 to 8 days following SAH, follows a similar time course and is frequently heralded by an increased leukocyte count in peripheral blood and a low-grade fever. It is probable that the 300-fold increase in IL-6 reflects a severe inflammation of the CNS. The interplay of CNS cytokines and monoamines has been studied only recently. Undoubtedly, a mutual influence of interleukin and monoamine levels exists, and fluctuations in their concentrations can be linked to clinical signs in experimental settings.

**Conclusions**

It appears from this study that SAH causes an immune activation in the CNS, entailing increased IL-6 and soluble IL-2R levels. The time course of IL-6 appearance and the fact that IL-6 levels were significantly increased in patients suffering from a delayed ischemic deficit are consistent with a hypothesis that deterioration following SAH may have immunological causes or reflect alterations of the immune system. However, further corroboration of such a hypothesis is needed, and further neuroimmunological research is necessary to analyze the regulation of IL-6 and soluble IL-2R secretion in CNS.

**References**

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