SHORT REPORT

Inflammatory cytokines in subarachnoid haemorrhage: association with abnormal blood flow velocities in basal cerebral arteries

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Abstract
Subarachnoidal release of inflammatory cytokines (interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α) was characterised in 35 patients with subarachnoid haemorrhage (SAH) and control subjects and compared with development of complicating haemodynamic abnormalities in basal cerebral arteries and clinical outcome. Serial analysis allowed the observation of a subacute response profile of these key mediators of inflammation in the subarachnoidal space. This compartmentalised inflammatory host response was closely associated in time and extent with development of increased blood flow velocities in the basal cerebral vessels as recorded by transcranial Doppler sonography. Moreover, intrathecal secretion of inflammatory cytokines was significantly increased in patients with poor clinical outcome. Together, these findings suggest a role of excessive compartmentalised inflammatory host response in pathogenesis of cerebrovascular complications after SAH.

Patients and methods

Subarachnoid haemorrhage (SAH) most commonly occurs when an aneurysm in a basal cerebral artery ruptures. Among patients who survive this event, the leading cause of death and disability is subsequent constriction of the large cerebral arteries causing delayed cerebral ischaemia, the “second stroke”.1,2 Monitoring of cerebral blood flow velocities (CBFVs) in the large pial arteries identifies patients with SAH with raised risk for ischaemic complications.3-5 In SAH, it has been shown in multiple studies that CBFVs on transcranial Doppler sonography are inversely related to vessel diameters on angiography.6 The non-invasive character of this technique enables serial investigations of developing haemodynamic abnormalities in an individual patient.

Earlier studies described inflammatory changes in SAH—that is, subarachnoidal and perivascular leucocytic infiltrates in the subarachnoidal space6-7 in relation to development of cerebral vasospasms.8,9 Moreover, the proinflammatory cytokines interleukin (IL)-1β, IL-6, and tumour necrosis factor (TNF)-α that orchestrate the cascade of inflammatory host response to infection and tissue damage10,11 have been detected in the CSF of patients with SAH.12-15 These earlier findings suggest an inflammatory pathogenesis of vasospasms in SAH. In this study, we tested the hypothesis that the extent and pattern of secretion of key mediators of inflammation IL-1β, IL-6, and TNF-α are associated with development of haemodynamic abnormalities in basal cerebral arteries and with clinical outcome.
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LONGITUDINAL STUDY OF SUBARACHNOID CYTOKINE SECRETION

Patients and Methods

Patients

Subarachnoid haemorrhage (SAH) was investigated in 80 patients (23 women and eight men), aged between 23 and 80 (median 40) years. These had lumbar puncture for initially suspected haemorrhage or meningitis, which was excluded by extensive clinical, neuroradiological, and laboratory investigation including CSF analysis.

MONITORING OF CBFS AND ASSESSMENT OF CLINICAL OUTCOME

Abnormalities of CBFVs were monitored by non-invasive transcranial Doppler sonography using a DWL Multidop X TCD device (DWL Sipplingen, FRG). Systolic CBFV values were recorded transtemporally in the proximal segments of the anterior, middle, posterior, and basilar cerebral arteries as described before.17 All patients had follow up CT at least twice to assess possible delayed cerebral ischaemia. In each patient, the score of the Glasgow outcome scale (GOS) was determined. This study was approved by the ethics committee of the Mannheim Clinic, University of Heidelberg.

MEASUREMENT OF PROINFLAMMATORY CYTOKINES

Plasma concentrations of proinflammatory cytokines (IL-1β, IL-6, and TNF-α) were quantified with sandwich immunoassays (R and D Systems, Minneapolis, MN, USA) as described before.14 Briefly, a monoclonal antibody specific for the antigen of interest was coated onto a 96 well microtitre plate. In a single step reaction, samples were then incubated in the microtitre plate together with a second horseradish peroxidase linked monoclonal antibody specific for a different epitope. After washing, the bound enzyme-antibody conjugate was measured enzymatically with tetramethylbenzidine as the substrate. Adsorbence was measured at 450 nm on an MR 4100 spectrophotometer (Dynatech, Burlington, MA, USA) using 630 nm as the reference wavelength. The lower limits of detection for IL-1β, IL-6, and TNF-α were 0.3 pg/ml, 0.3 pg/ml, and 4.4 pg/ml, respectively.

STATISTICAL ANALYSIS

Results are expressed as means (SEM). Statistical analysis involved a Mann-Whitney U test and calculation of Spearman’s correlation coefficient.

Results

CHARACTERISATION OF INTRATHECAL RELEASE OF PROINFLAMMATORY CYTOKINES IN SAH

Concentrations of IL-1β, IL-6, and TNF-α in the CSF of control subjects were 0.00 (0.00) pg/ml, 0.0004 (0.0001) ng/ml, and 0.18 (0.16) pg, respectively. Compared with these normal values, CSF concentrations of proinflammatory cytokines in patients with SAH were massively increased in SAH (fig 1)—for example, CSF concentrations of IL-6 were increased up to 10 000-fold. Longitudinal analysis showed a subacute response pattern for these cytokines, peaking between days 5 and 9 and declining thereafter (fig 1). In SAH, concentrations of IL-1β and IL-6 were significantly lower in plasma than in CSF, showing the intrathecal origin of these mediators. Plasma and CSF concentrations of IL-1β and IL-6 at day 5 were 2.21 (0.70) v 5.78 (0.83) pg/ml (p<0.0001) and 0.01 (0.04) v 9.15 (2.26) ng/ml (p<0.0001), respectively. By contrast, serum and CSF concentrations of TNF-α did not differ significantly (3.72 (0.73) v 3.62 (0.50) pg/ml; p=0.69). Samples of CSF obtained from extra-ventricular drainage (EVD) in 77% of the patients and by lumbar puncture in 23% did not differ in cytokine concentrations (data not shown).

ALTERATIONS OF CBFS AND ASSOCIATION WITH SUBARACHNOID CYTOKINE SECRETION

Mean systolic CBFV subacutely increased during the study period of 11 days, peaking between days 7 and 11 when values reached a plateau (fig 2). Abnormalities were most pronounced in the middle cerebral arteries. Interestingly, changes in CBFVs paralleled those of CSF concentrations of inflammatory cytokines (figs 1 and 2). The response in the CSF, however, slightly preceded those of the haemodynamic abnormalities, peaking between days 5 and 9. We subdivided patients into two subpopulations with higher (>210 cm/s) mean values, CSF concentrations of proinflammatory cytokines (fig 1). * p<0.05.
Figure 2 Kinetics of changes in CBFV (mean (SEM)) in intracranial basal arteries as determined by transcranial Doppler sonography (n=20). Reference values as derived from our earlier readings in 50 normal volunteers were 94.5 (1.9) cm/s (middle cerebral artery, MCA), 76.4 (2.4) cm/s (anterior cerebral artery, ACA), and 34.4 (1.7) cm/s (basilar artery, BA).  

highly increased CBF cytokines\(^{17}\) or lower CBFVs (<210 cm/s). Development of CBFVs>210 cm/s (n=22) was associated with significantly increased CSF concentrations of IL-1\(\beta\), IL-6, and TNF-\(\alpha\) compared with presence of CBFV<210 cm/s (n=13, fig 1).

Clinical outcome and association with subarachnoid cytokine secretion

The GOS scores were 1 in 33%, 2 in 27%, 3 in 9%, 4 in 6%, and 5 in 24% of the patients. Concentrations of IL-6 at day 5 were significantly increased in patients with poor (GOS 1–2) compared with those with moderate or good (GOS 3–5) outcome, whereas concentrations of IL-1\(\beta\) and TNF-\(\alpha\) exhibited a non-significant trend towards increased concentrations in the subpopulation with poor outcome (fig 3). Follow up CT showed signs of newly developed cerebral ischaemic injury in 46% of the patients with SAH. These patients exhibited a non-significant trend towards increased CSF concentrations of IL-6 at day 5 (12.11 (3.51) ng/ml v 6.51 (2.25) ng/ml, p=0.08).

Concentrations of IL-1\(\beta\) (r=0.87, NS), IL-6 (r=0.19, NS), and TNF-\(\alpha\) (r=−0.16, NS) did not correlate with Hunt and Hess scores. Hunt and Hess scores were not significantly different in patients with higher or lower CBFVs (3.42 (0.25) v 3.33 (0.36), p=0.87). As expected, the scores, however, correlated with GOS (r=−0.59, p<0.005). Interestingly, GOS scores were not significantly different in patients with lower or higher CBFVs (2.73 (0.52) v 2.43 (0.33), NS) in this study. Neither sex nor age were significantly associated with CSF cytokine concentrations. Thus, age did not correlate with concentrations of IL-1\(\beta\) (r=0.40, NS), IL-6 (r=0.23, NS), or TNF-\(\alpha\) (r=0.14, NS). Similarly, concentrations of IL-1\(\beta\) (0.95 (1.45) v 5.03 (0.98) pg/ml, NS), IL-6 (11 763.85 (3134) v 8291.33 (2983) pg/ml, NS) or TNF-\(\alpha\) (3.85 (0.87) v 3.48 (0.62) pg/ml, NS) were not significantly different in male or female patients. Moreover, age was not significantly increased in patients with higher CBFVs compared with those with lower CBFVs (57.33 (2.10) v 57.83 (5.04), NS) and mean CBFVs in the middle cerebral artery were not significantly different in male or female patients (186.08 (16.31) v 325.00 (13.61) cm/s, NS). Finally, age did not correlate with GOS scores (r=−0.32, NS) and these scores were not significantly different in male or female patients (2.31 (0.46) v 2.80 (0.35), NS).

Discussion

Vasospasms and secondary cerebral ischaemia are the most important complications of SAH. This study showed that the release of IL-1\(\beta\), IL-6, and TNF-\(\alpha\) in the subarachnoidal space of patients with SAH is associated with development of increased CBFVs in basal cerebral arteries suggesting a pathophysiological role of excessive subarachnoidal inflammatory host response in haemodynamic complications after SAH.

Secretion of these inflammatory cytokines gradually increased, reaching peak concentrations at days 5–9. Peak concentrations were up to 10 000-fold increased, in the range previously detected in bacterial meningitis.\(^{17}\) Interestingly, the haemodynamic abnormalities in recorded basal cerebral arteries of patients with SAH were characterised by a similar subacute temporal profile, only slightly lagging behind the response of the inflammatory cytokines. Release of inflammatory cytokines in the subarachnoidal space was significantly increased in the subpopulation of patients with SAH with higher CBFVs. These findings are consistent with a recent report of higher IL-6 concentrations in five patients with clinical signs of vasospasms after SAH.\(^{18}\) Interestingly, CSF concentrations of IL-6 were significantly increased in patients with SAH with poor clinical outcome, further delineating the clinical relevance of intrathecal cytokine secretion in SAH.

Such pathophysiological importance of compartmentalised cytokine release for development of cerebrovascular complications after SAH could also explain the earlier observations of severe vasospasms in experimental subarachnoidal inflammation\(^{19}\) or in bacterial meningitis.\(^{17}\) Both conditions are characterised by massive cytokine release\(^{18}\) but by an obvious absence of erythrocytes or platelets that are generally considered as key cells in pathophysiology of vasospasms after SAH.

The triggers for the pronounced inflammatory host response in the subarachnoidal space of patients with SAH are unknown. One mechanism could be complement activation, a strong inflammatory stimulus, by osmotically induced disruption of erythrocytes. This is
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suggested by in vitro findings of a complement mediated haemolysis of erythrocytes in subarachnoid blood clot or by raised CSF concentrations of complement factors in SAH.  

Subarachnoid release of inflammatory cytokines could contribute to cerebral vasospasms in several ways: Once released in the subarachnoidal space, these mediators (17–26 kD) could easily access the smooth muscle cells of contiguous basal arteries from their adventitial side as even much larger molecules (for example, horseradish peroxidase, MW 40 kD) pass from the cisterna magna through the vessel wall to the basal membrane within minutes. This is possible because the surface of the pial cerebral arteries is, exceptionally, not confined by collagen or fibroblasts but is in direct contact with the nourishing CSF. Cytokines may also modulate vessel tone in an indirect manner—for example, by induction of synthesis of vasoconstrictive factors such as ET-1 or by induction of adhesion molecule expression responsible for focal leucocyte recruitment. It has, however, to be noted that further factors may play a part in pathophysiology of cerebral artery narrowing after SAH and multiple therapeutic approaches to cerebral vascular complications after SAH are currently explored.

Although we did not find different cytokine concentrations in CSF derived from EVD or by lumbar puncture, effects of the different origin of CSF on these measurements cannot be excluded.

In conclusion, although the clinical and laboratory correlations found cannot prove causal relations, this study supports the hypothesis that an overwhelming inflammatory host response in the subarachnoidal space of patients with SAH plays a central part in pathogenesis of vasospasms and subsequent cerebral ischaemia. As a therapeutic consequence of these findings, we speculate that early subarachnoidal administration of anti-inflammatory or anticytokine strategies could prevent haemodynamic complications after SAH.

We are grateful to Mrs M Garcia-Knapp for her secretarial assistance in the preparation of the manuscript.

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*J Neurol Neurosurg Psychiatry* 2001 70: 534-537
doi: 10.1136/jnnp.70.4.534

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