Levels of Anti-Inflammatory Cytokines and Neurological Worsening in Acute Ischemic Stroke

Nicolás Vila, MD; José Castillo, MD; Antonio Dávalos, MD; Anna Esteve, PhD; Anna M. Planas, PhD; Ángel Chamorro, MD

Background—Mechanisms involved in stroke progression are incompletely understood. Ischemic brain injury is characterized by acute local inflammatory response mediated by cytokines. Anti-inflammatory cytokines act in a feedback loop to inhibit continued proinflammatory cytokine production. We assessed the implication of interleukin (IL)-10 and IL-4 in deteriorating ischemic stroke.

Methods—Two hundred thirty-one patients with ischemic stroke within the first 24 hours from onset were included. Neurological worsening was defined when the Canadian Stroke Scale score fell at least 1 point during the first 48 hours after admission. Anti-inflammatory cytokines were determined in plasma obtained on admission.

Results—Eighty-three patients (35.9%) worsened within the first 48 hours after stroke onset. Significantly lower concentrations of IL-10 were found in patients with neurological worsening (P<0.05), but IL-4 levels were similar in patients with or without deterioration. Lower plasma concentrations of IL-10 (<6 pg/mL) were associated with clinical worsening on multivariate analysis (odds ratio = 3.1, 95% CI = 1.1 to 8.9) independently of hyperthermia, hyperglycemia, or neurological condition on admission. Further analysis disclosed that early worsening was independently associated with lower IL-10 plasma levels in patients with subcortical infaracts or lacunar stroke but not in patients with cortical lesions.

Conclusions—Anti-inflammatory cytokine IL-10 is associated with the early clinical course of patients with acute ischemic stroke, especially in patients with small vessel disease or subcortical infarctions. (Stroke. 2003;34:671-675.)

Key Words: cytokines ■ inflammation ■ interleukin-4 ■ interleukin-10 ■ neuroprotection ■ stroke, ischemic

One third of patients with acute ischemic stroke develop early neurological worsening, a situation associated with increased mortality and long-term functional disability.1,2 The underlying basic mechanisms involved are not completely understood, although biochemical factors have been suggested.1,2 Cerebral ischemia evokes an inflammatory response characterized by activation and release of cytokines, chemokines, endothelial-leukocyte adhesion molecules, and proteolytic enzymes that exacerbate tissue damage.3,4 Increased production of proinflammatory cytokines and chemokines has been detected in experimental models of brain ischemia as well in patients with acute stroke.3,4 Moreover, increased levels of proinflammatory cytokines is related to a greater extent of cerebral infarct and poorer clinical outcome in patients with ischemic stroke.1–6 Ischemic tissue damage can be reduced in experimental models with a variety of anti-inflammatory agents including antibodies against adhesion molecules, neutrophil depletants, inhibitors of proinflammatory cytokines, and anti-inflammatory cytokines.7–10 Interleukin (IL)-10 and IL-4 are anti-inflammatory molecules, mainly secreted by lymphocytes and monocytes/macrophages, that block proinflammatory actions.11 IL-10 and IL-4 may provide a negative feedback mechanism to limit production of proinflammatory cytokines in ischemic stroke. Administration of IL-109,10 or gene transfer11 in experimental models of focal brain ischemia reduces infarct volume, suggesting that it may have neuroprotective effects.6–10 In patients with acute stroke, a transient increase in IL-10 concentrations in plasma, cerebrospinal fluid (CSF), and blood mononuclear cells have been detected.12–14 Moreover, low levels of IL-10 have been associated with an unstable clinical course in patients with angina.15 Therefore, in this prospective study we evaluated whether plasma concentrations of anti-inflammatory cytokines (IL-10 and IL-4) are associated with early worsening of neurological symptoms in patients with acute ischemic stroke.

Methods

From a cohort of 249 consecutive patients with first-ever acute ischemic stroke admitted within 24 hours of the onset of symptoms...
in a prospective investigation evaluating factors related to stroke deterioration, a group of 231 patients who had available stored plasma samples were evaluated in the present secondary analysis. To avoid the confounding effect on inflammatory markers, subjects with inflammatory or infectious diseases, cancer, hematological diseases, or severe renal or liver failure were not included in the study. The protocol was approved by the local Ethics Committee, and the methodology was described previously. Briefly, patients were admitted to neurological wards and managed according to evidence-based stroke guidelines. Thrombolysis, hemodilution, corticosteroids, or nimodipine were not permitted. Antiplaquette drugs were used in atherothrombotic and lacunar infarcts and anticoagulants in suspected cardioembolic infarcts when cranial CT scan and clinical examination did not suggest a large cerebral lesion. Stroke subtype was classified according to TOAST definitions. Stroke severity examination did not suggest a large cerebral lesion. Stroke subtype suspected cardioembolic infarcts when cranial CT scan and clinical

TABLE 1. Main Characteristics of Study Population

<table>
<thead>
<tr>
<th>Neurological Worsening</th>
<th>Stable Course</th>
</tr>
</thead>
<tbody>
<tr>
<td>(n=83)</td>
<td>(n=148)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>9 (59)</td>
</tr>
<tr>
<td>Age, y</td>
<td>8.5 (8.6)</td>
</tr>
<tr>
<td>Admission delay, h**</td>
<td>0.2 (6.2)</td>
</tr>
<tr>
<td>Admission CSS</td>
<td>0.2 (1.9)</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>60 (26.6)</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>1.7 (16.8)</td>
</tr>
<tr>
<td>Serum glucose, mg/dL*</td>
<td>29.8 (54.4)</td>
</tr>
<tr>
<td>Body temperature, °C*</td>
<td>7.8 (0.6)</td>
</tr>
<tr>
<td>Leukocyte count, ×10^9/L</td>
<td>0.9 (1.6)</td>
</tr>
<tr>
<td>Early CT signs**</td>
<td>4 (89.2)</td>
</tr>
<tr>
<td>Infarct volume on day 4–7, cc*</td>
<td>63.7 (54.1)</td>
</tr>
<tr>
<td>Infarct topography†</td>
<td></td>
</tr>
<tr>
<td>Cortical</td>
<td>32 (28.6)</td>
</tr>
<tr>
<td>Subcortical</td>
<td>21 (21)</td>
</tr>
<tr>
<td>Stroke etiology</td>
<td></td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>30 (36.1)</td>
</tr>
<tr>
<td>Lacunar</td>
<td>7 (8.4)</td>
</tr>
<tr>
<td>Atherosclerotic</td>
<td>35 (42.2)</td>
</tr>
<tr>
<td>Undetermined</td>
<td>11 (13.3)</td>
</tr>
</tbody>
</table>

Numbers represent mean (SD) or percentage as appropriate. CSS indicates Canadian Stroke Scale; SBP, systolic blood pressure; DBP, diastolic blood pressure.

*P<0.001; ***P<0.05.
†Restricted to 176 patients with cortical or subcortical lesions.

For cytokine determination, blood samples of patients were collected on admission in tubes with potassium edetate, centrifuged at 3000g for 5 minutes, and immediately frozen and stored until analysis (5 to 7 years at −80°C). Mean admission delay from stroke onset was 8.2±5.7 hours (range=1.5 to 23 hours). Samples were collected within 12 hours in 80% of patients and within 6 hours in 50%. Samples were also obtained in 43 patients admitted without neurological disorders (29 men and 21 women; mean age 56±17 years). Plasma concentrations of IL-10 and IL-4 were measured with a commercially available quantitative immunoassay (QuantiKine) obtained from R&D Systems. The minimum detectable doses for human IL-10 and IL-4 provided by the manufacturer were 0.5 and 10 pg/mL, respectively. Cytokine determinations were done blinded to clinical and radiological data.

For statistical analyses, the χ2 test, the Student t test, and the Mann-Whitney test were used as appropriate. Stroke deterioration was assessed by stepwise logistic regression analysis entering into the model all variables with probability value ≤0.10 on univariate testing, which included IL-10, body temperature, fasting serum glucose, fibrinogen, total leukocyte count, baseline CSS, admission delay, and presence of early infarct signs on brain CT scan. Continuous variables without normal distribution were analyzed after their logarithmic transformation. Cutoff values for plasma IL-10 were calculated as described by Robert et al given the different distribution of IL-10 according to outcome groups. Odds ratios and 95% confidence intervals were calculated from β coefficients and their SEs. A probability value <0.05 was established as statistically significant.

Results

In 69 patients (29.9%), no changes were detected in the CSS score. Seventy-nine patients (34.2%) improved their CSS score, as follows: 54 (23.4%) by 1 point, 21 (9.1%) by 2 points, and 4 by ≥3 points. Eighty-three (35.9%) patients worsened their CSS score within the first 48 hours after stroke onset, as follows: 41 (17.7%) by 1 point, 24 (10.4%) by 2 points, and 18 (7.8%) by ≥3 points. The main characteristics of the studied population are shown in Table 1. Patients with early stroke deterioration had a slightly longer delay to hospital admission and higher baseline glucose, fibrinogen, and body temperature compared with patients with a stable clinical course. However, the two clinical groups disclosed similar stroke subtypes and degree of neurological impairment on admission.

Although plasma concentrations of IL-10 were not different between control group (5.9±1.6 pg/mL) and stroke patients (6.0±2.2 pg/mL), IL-10 levels were significantly higher in those stroke patients who remained stable or improved during the first 48 hours than in patients with clinical deterioration (6.2±2.2 pg/mL versus 5.6±2.0 pg/mL; P<0.05) (Figure 1). However, concentrations of IL-4 were similar in both outcome groups (21.2±5.3 versus 20.3±5.5 pg/mL).

As shown in Table 2, the following variables remained on multivariate analysis independently associated with clinical worsening: IL-10 <6 pg/mL in plasma, body temperature, admission CSS, and serum glucose. Overall, levels of IL-10 were similar in patients with cortical and subcortical infarcts (Table 3). However, significantly lower IL-10 levels were

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detected in patients with subcortical infarcts who deteriorated compared with patients with subcortical infarcts who remained stable or improved (5.3 ± 1.0 versus 6.1 ± 0.5 pg/mL; P < 0.05). Behavior of IL-10 levels was similar in cortical infarcts, but differences did not achieve statistical significance. In addition, similar levels of IL-4 were found in patients with and without worsening in both cortical and subcortical infarcts.

Table 3 also shows that levels of IL-10 were not different between stroke subtypes. Figure 2 represents IL-10 levels and stroke progression in relation to stroke subtypes. Levels of IL-10 were lower in patients with deterioration in all subgroups. However, significant differences between plasma IL-10 levels and stroke worsening were only detected in patients with lacunar infarcts. Those patients with lacunar infarcts that deteriorated had lower levels of IL-10 compared with those with infarcts that remained stable or improved (5.3 ± 1.2 versus 6.5 ± 0.9 pg/mL; P < 0.01). No differences in levels of IL-4 were found in patients with and without worsening according to stroke subtypes.

Finally, in 61 patients with nonlacunar infarcts and subcortical topography, small but significant lower plasma concentrations of IL-10 were detected in subjects with early neurological deterioration (n = 16) compared with those with stable or improved course (n = 45) (5.2 ± 2.4 versus 5.8 ± 2.0 pg/mL; P = 0.05).

Discussion
IL-10 is one of the anti-inflammatory cytokines that may regulate the complex network of reactions that occur in acute cerebral ischemia. In the present study, lower levels of IL-10 in plasma measured within 24 hours from stroke onset were significantly associated with early deterioration of neurological symptoms in patients with ischemic stroke. This relation was independent of other well-known predictors of clinical worsening such as clinical severity on admission, hyperglycemia, and early CT signs of infarction or hyperthermia. In humans, increased concentrations of IL-10 are found shortly after stroke onset in CSF and plasma, reaching the highest level between days 3 and 7.12,14 In addition, patients with acute stroke have increased IL-10 secreting monocytes in peripheral blood compared with controls.13 Contrarily, we did not find differences in IL-4 levels in patients with or without neurological deterioration. These results suggest that IL-4, despite its inhibitory capacity on proinflammatory cytokines, is less important than IL-10 in acute ischemic stroke. In agreement with this finding, Pelidou et al13 also failed to demonstrate differences in IL-4–secreting monocytes in patients with stroke compared with controls.

The present study also shows an association between lower levels of IL-10 with neurological deterioration in subcortical and lacunar infarcts. These findings give further support to previous observations linking cytokines with early motor impairment in patients with lacunar stroke.6 Previously, we had reported that lacunar stroke might result from genetic predisposition.
susceptibility to inflammation-mediated damage in concert with atherosclerotic risk factors. A relationship between proinflammatory cytokines, excitatory amino acids, and neurological worsening in lacunar infarcts were also found. Increase in infarct volume, as demonstrated with diffusion MRI, has been proposed as the main cause of neurological deterioration in lacunar infarcts as the likely result of delayed excitotoxic and inflammatory neuronal death. Thus, if these findings are validated, patients with lacunar infarcts would represent ideal candidates to participate in clinical trials of neuroprotection using anti-inflammatory drugs or inhibitors of excitotoxicity. In addition, these biological markers might be useful in the future to detect those patients with lacunar stroke at risk of motor deterioration, especially when this stroke subtype is the major cause of progressive motor deficits.

A direct implication of IL-10 in the pathophysiology of ischemic neurological deterioration cannot be extrapolated from our results as the levels of anti-inflammatory cytokines were not determined exactly at the moment of neurological deterioration. As well, these results must be interpreted with caution given that plasma concentrations of cytokines do not necessarily reflect the activity of cytokines within the central nervous system. Also, we recognize that we have not explored the potential effect of cytokines on late worsening. However, a body of experimental evidence implicates this anti-inflammatory cytokine in the downregulation of deleterious actions of proinflammatory cytokines. IL-10 inhibits monocyte/macrophage synthesis of IL-6 and tumor necrosis factor-α by blocking gene transcription and downregulates release of intercellular adhesion molecule-1 (ICAM-1) and matrix metalloproteinase (MMP). Animals deficient in IL-10 gene exhibited larger infarcts; increased neutrophil infiltration; and raised levels of tumor necrosis factor-α, ICAM-1, MMP-2, and MMP-9. Additionally, IL-10 may have neuroprotective activities resulting from other non–anti-inflammatory actions. This cytokine regulates apoptotic proteins detected in CSF after human brain ischemia, modulates neuronal vulnerability to excitotoxic ischemic damage, and inhibits the inducible form of NO synthase (iNOS). IL-10–deficient mice also developed increased levels of iNOS compared with the wild type. Levels of excitotoxic amino acids, NO products, proinflammatory cytokines, and MMP in plasma or in CSF have also been related with clinical outcome in human stroke. Contrarily, in hemorrhagic stroke it seems that anti-inflammatory cytokines do not play the same role as in cerebral ischemia. Recently, levels of IL-10 and IL-4 were found to be unrelated to clinical outcome in patients with cerebral hemorrhages.

If levels of IL-10 are markers of neurological deterioration, an important issue is to determine why stroke patients may exhibit a different anti-inflammatory profile. Recently, several functional IL-10 gene polymorphisms have been described in the population. Those patients with a specific IL-10 gene polymorphism, that determine low levels of IL-10, may be at risk to develop unstable vascular syndromes. A recent work demonstrated a relationship between low levels of IL-10 in plasma and the subsequent risk of stroke and also with mortality when the stroke occurs. If this association is confirmed, those subjects genetically predisposed might be treated with exogenous IL-10 to prevent stroke and clinical worsening in acute stroke. Recombinant human IL-10 administered by intravenous injection in healthy volunteers is well tolerated and safe. Thus, in addition to potential indications of IL-10 in chronic inflammatory diseases under current evaluation, this study gives additional evidence that IL-10 may have a potential role as neuroprotectant in acute vascular syndromes.

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References


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