Short Communications

Inflammation and Stroke
The Leiden 85-Plus Study

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Background—Experimental evidence indicates that interleukin-10 (IL-10) deficiency is associated with the development of cardiovascular and cerebrovascular disease. We analyzed the relation between low IL-10 production levels, history of stroke, and incident fatal stroke.

Summary of Report—All 85-year-old inhabitants of Leiden, Netherlands (n=599) were visited at their place of residence (response rate, 87%). Production levels of the anti-inflammatory cytokine IL-10 were assessed in a whole blood assay whereby lipopolysaccharide was used as a stimulus. Plasma concentrations of C-reactive protein (CRP) were also used as a marker of inflammation. A history of stroke was obtained at baseline (prevalence, 10%). The number of fatal strokes was prospectively obtained for a median follow-up of 2.6 years (incidence, 1.82 per 100 person-years at risk). Subjects with a history of stroke had significantly lower median IL-10 production levels at baseline than subjects without stroke (558 versus 764 pg/mL; P<0.05). They also had significantly higher median CRP concentrations (6 versus 3 mg/L; P<0.05). The odds ratio for a history of stroke increased to 2.30 (95% CI, 1.12 to 4.72) over strata representing decreasing production levels of IL-10. The relative risk for incident fatal stroke was 2.94 (95% CI, 1.01 to 8.53) when we compared subjects with low or intermediate baseline IL-10 production levels to those with high production levels of IL-10.

Conclusions—Our data support the hypothesis that subjects with low IL-10 production levels have an increased risk of stroke. (Stroke. 2002;33:1135-1138.)

Key Words: C-reactive protein • cytokines • inflammation • interleukin-10 • stroke

Accumulating evidence suggests that inflammation plays an important role in the development of cardiovascular and cerebrovascular disease.1-3 Markers of inflammation, such as C-reactive protein (CRP),1,2 and pro-inflammatory cytokines are associated with stroke.4 Interleukin-10 (IL-10) is a centrally operating anti-inflammatory cytokine that plays a crucial role in the regulation of the innate immune system. It has strong deactivating effects on the inflammatory host response and potently inhibits the production of proinflammatory cytokines.5 Animal models investigating the protective role of IL-10 in atherosclerosis show that IL-10–deficient mice have a high susceptibility to atherosclerosis.6 Moreover, IL-10–deficient mice have an increased stroke lesion size after ligation of the mid-cerebral artery,7 whereas rats treated with IL-10 have a decreased stroke lesion size.8

In this study we tested the hypothesis that a proinflammatory cytokine response predisposes to stroke. We therefore analyzed the association between low IL-10 production levels and a history of stroke. We also determined the association between low IL-10 production levels measured at baseline and incident fatal stroke.

Subjects and Methods
Between September 1, 1997, and September 1, 1999, 705 inhabitants of Leiden, Netherlands, reached the age of 85 years and were eligible to participate in the Leiden 85-Plus Study. There were no selection criteria for health or demographic characteristics. The Medical Ethical Committee of the Leiden University Medical Center approved the study. Fourteen inhabitants died before they could be enrolled. The response rate was 87%; a total of 599 subjects (397 women, 202 men) participated. There were no significant differences for various demographic characteristics between the 599 respondents and the source population.

The World Health Organization definition of stroke of “rapidly developing clinical signs of focal (at times global) disturbance of cerebral functioning lasting >24 hours” was used to identify subjects with a history of stroke. The subjects’ general practitioners or treating (nursing home) physicians were interviewed to obtain a complete medical history, including a history of stroke. The advantage of using general practitioners to obtain a history of stroke was that subjects with a history of stroke, who were not hospitalized, were also included in the study. All subjects were followed up for mortality until September 1, 2001. The primary and secondary causes of death were obtained from subjects’ general practitioners or treating physicians. Fatal stroke was classified according to the International Statistical Classification of Diseases, 10th Revision codes I60 to I69.

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IL-10 production in lipopolysaccharide-stimulated whole-blood samples varies between individuals. This interindividual variation has a strong genetic basis. Family studies of first-degree relatives and analysis of quantitative IL-10 production in humans are derived from heritable factors. The innate IL-10 production was assessed with an ex vivo whole-blood assay. The complete methods by which whole-blood samples were simulated with 10 ng/mL of lipopolysaccharide have been described elsewhere. Unstimulated baseline samples were obtained to serve as a control for contamination. Subjects with detectable tumor necrosis factor-α (TNF-α) concentrations under unstimulated conditions (TNF-α >100 pg/mL) were therefore excluded from further analysis. Plasma levels of CRP were measured with the use of a fully automated Hitachi 911 device.

Subjects were classified as having diabetes when they met at least 1 of the following criteria: (1) history of diabetes, obtained from the subject’s general practitioner or treating physician; (2) use of sulfonylureas, biguanides, or insulin, obtained from the subject’s pharmacist; or (3) nonfasting glucose concentrations of ≥11.1 mmol/L. Subjects were classified as having hypertension when they met at least 1 of the following criteria: (1) history of hypertension; (2) use of β-blockers, angiotensin-converting enzyme inhibitors, thiazide diuretics, or calcium antagonists, obtained from the subject’s pharmacist; or (3) a diastolic blood pressure of ≥95 mm Hg or a systolic blood pressure of ≥180 mm Hg. Subjects were classified as having cardiovascular disease when they met at least 1 of the following criteria: (1) history of myocardial infarction, angina pectoris, arterial surgery, or intermittent claudication; or (2) signs of myocardial infarction or myocardial ischemia recorded on the ECG, which was obtained in all subjects. Finally, information regarding use of nonsteroidal anti-inflammatory drugs (NSAIDs), including aspirin, was obtained from the subject’s pharmacist.

Data Analysis
Data are presented as median with corresponding 95% CIs for the median, representing the range of values that includes the "true" median. The nonparametric Mann-Whitney test was used because IL-10 production levels and CRP concentrations were not normally distributed and were skewed to the right. The production levels of IL-10 were grouped into 3 equal strata representing decreasing IL-10 production levels (Figure 1; †Values are medians and corresponding 95% CIs. The nonparametric Mann-Whitney test was used because IL-10 production levels and CRP concentrations were not normally distributed and were skewed to the right. The production levels of IL-10 were grouped into 3 equal strata representing decreasing IL-10 production levels (Figure 1; †Values are medians and corresponding 95% CIs.

Cross-Sectional Study
The Table shows clinical and inflammatory characteristics of the subjects at baseline. Subjects with a history of stroke had lower median IL-10 production levels and higher median CRP concentrations than subjects without stroke (IL-10 production levels, 558 versus 764 pg/mL [P=0.047]; CRP, 6 versus 3 mg/L [P=0.004]). We found a dose-response relationship between IL-10 production levels and subjects without a history of stroke, subjects with 1 stroke (n=45), and 10 subjects with ≥2 strokes (IL-10 production levels, 764, 597, and 542 pg/mL, respectively [P for trend=0.06]). In an additional analysis, we excluded subjects who used NSAIDs (n=153). The IL-10 production levels and plasma CRP concentrations in subjects with a history of stroke compared with those without stroke remained similar (IL-10 production levels, 545 versus 756 pg/mL [P=0.12]; CRP, 8 versus 3 mg/L [P=0.003]).

The odds ratio for a history of stroke, adjusted for type 2 diabetes, hypertension, use of NSAIDs, and cardiovascular disease, increased to 2.30 (95% CI, 1.12 to 4.72) over strata representing decreasing production levels of IL-10 (Figure 1; †Values are medians and corresponding 95% CIs.

Follow-Up Study
In total, 147 subjects died during a median follow-up of 2.6 years. Twenty-six of them suffered a fatal stroke (incidence, 1.82 per 100 person-years at risk; 95% CI, 1.12 to 2.53). Eight of the 26 subjects with fatal stroke had a history of stroke at baseline. Only 4 of the 183 subjects (2.1%) with high IL-10 production levels suffered a fatal stroke, whereas 12 of the 185 subjects (6.5%) with intermediate IL-10 levels died of stroke at baseline. Only 4 of the 183 subjects (2.1%) with high IL-10 production levels suffered a fatal stroke, whereas 12 of the 185 subjects (6.5%) with intermediate IL-10 levels died of stroke at baseline. Only 4 of the 183 subjects (2.1%) with high IL-10 production levels suffered a fatal stroke, whereas 12 of the 185 subjects (6.5%) with intermediate IL-10 levels died of stroke at baseline.

Clinical and Inflammatory Characteristics in Relation to History of Stroke

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>Absent (n=496)</th>
<th>Present (n=55)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>85</td>
<td>85</td>
<td></td>
</tr>
<tr>
<td>Women</td>
<td>332 (67%)</td>
<td>36 (65%)</td>
<td>0.83</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>78 (16%)</td>
<td>11 (20%)</td>
<td>0.41</td>
</tr>
<tr>
<td>Hypertension</td>
<td>277 (56%)</td>
<td>38 (69%)</td>
<td>0.06</td>
</tr>
<tr>
<td>Cardiovascular disease</td>
<td>285 (57%)</td>
<td>36 (65%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Use of NSAID†</td>
<td>125 (25%)</td>
<td>28 (51%)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Inflammatory characteristics†

| IL-10, pg/mL              | 764 (727–803) | 558 (465–817) | 0.047 |
| CRP, mg/L                 | 3 (3–4)       | 6 (3–9)       | 0.004 |

*Including use of aspirin.
†Values are medians and corresponding 95% CIs.
production levels and 10 of the 183 subjects (5.5%) with low IL-10 production levels suffered a fatal stroke. Figure 2 shows the cumulative mortality for stroke, of the 551 participating subjects, over strata of IL-10 production and strata of CRP. The highest cumulative mortality for stroke was present for those with low or intermediate IL-10 production levels \((P=0.06, \text{Cox regression})\) and those with high or intermediate CRP \((P=0.095, \text{Cox regression})\).

The crude relative risk for incident fatal stroke was 2.94 (95% CI, 1.01 to 8.53) when we compared subjects with low or intermediate production levels of IL-10 measured at baseline to those with high IL-10 production levels. Each 500-pg/mL increase of IL-10 production corresponded to a 36% decrease in mortality due to stroke (risk ratio, 0.64; 95% CI, 0.39 to 1.00). The results remained similar after adjustment for sex, type 2 diabetes, hypertension, use of NSAIDs, and cardiovascular disease (risk ratio, 0.67; 95% CI, 0.41 to 1.00). The median IL-10 production level, measured at baseline, was lower in those with a fatal stroke \((n=26)\) than in those without a fatal stroke \((n=525)\) (715 versus 764 pg/mL; \(P=0.07\)). The median CRP concentration, measured at baseline, was higher in those with a fatal stroke than in those without a fatal stroke \((5 \text{ versus } 3 \text{ mg/L}; P=0.14)\). To rule out the possibility that low IL-10 production levels were markers for intercurrent fatal disease, we excluded those subjects who died during the first half year of follow-up \((n=11)\), leaving 540 subjects in the analysis. Adjustments were made for diabetes, hypertension, use of NSAIDs, history of stroke, and presence of cardiovascular disease at baseline with the use of Cox regression. After the age of 85.5 years, the adjusted relative risk for incident fatal stroke \((n=25)\) was 3.63 (95% CI, 1.08 to 12.21) when we compared subjects with low or intermediate production levels of IL-10 measured at baseline to those with high IL-10 production levels.

**Discussion**

This analysis of the Leiden 85-Plus Study shows that low IL-10 production levels are associated with both a history of stroke, in the cross-sectional study, and increased mortality due to stroke, as obtained in the prospective follow-up study. These associations persisted after adjustment for known risk factors for stroke. In accord with earlier clinical studies, we also showed that high CRP was associated with stroke.\(^1\)\(^2\) Our findings extend data from animal models that showed that IL-10 deficiency predisposes to atherosclerosis\(^6\) and increases stroke lesion size.\(^7\)\(^8\)
Since both our cross-sectional and longitudinal data showed an association between low IL-10 production levels and an increased risk of stroke, it is tempting to speculate that the association between low IL-10 production levels and stroke is causal. Furthermore, we have previously shown associations between innate IL-10 production and multiple sclerosis\(^\text{10}\) and used a family design in which the cytokine response of the patient was estimated in first-degree relatives.\(^\text{9–11}\) Additionally, the genetic basis of IL-10 production favors a causal interpretation of the association. Finally, other findings suggest that the cytokine response in whole blood induces the same effects in the brain across the blood-brain barrier.\(^\text{13}\)

In our study both high CRP and low IL-10 production are associated with stroke. We believe that CRP and IL-10 at least partly represent the effect of an inflammatory response on stroke. Studies of cerebral ischemia emphasize the relevance of an inflammatory response in regard to lesion size, in which IL-10 is a key regulator.\(^\text{5,7,8}\) IL-10 is a powerful suppressor of the immune response, produced by T cells, B cells, monocytes, macrophages, and microglia.\(^\text{5,14}\) It inhibits proinflammatory cytokines such as TNF-\(\alpha\) and IL-6.\(^\text{5}\) It may also inhibit CRP, since it has been suggested that IL-6 partly regulates CRP production.\(^\text{15}\) Moreover, IL-10 limits the size of ischemic brain damage occurring after occlusion of cerebral arteries.\(^\text{8}\) IL-10 could therefore represent a potential therapeutic agent for inflammatory diseases such as atherosclerosis and stroke.

In summary, low IL-10 production levels and high plasma CRP concentrations are associated with an increased risk of stroke, obtained at baseline and during follow-up. These findings support the hypothesis that a proinflammatory response predisposes to stroke.

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References
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