Inflammation in High-Grade Carotid Stenosis
A Possible Role for Macrophages and T Cells in Plaque Destabilization

Sebastian Jander, MD; Matthias Sitzer, MD; René Schumann; Michael Schroeter, MD; Mario Siebler, MD; Helmuth Steinmetz, MD; Guido Stoll, MD

Background and Purpose—Inflammatory mechanisms have been implicated in the pathogenesis of atherosclerosis. In this study, we investigated whether the extent of inflammatory infiltration in high-grade carotid artery (ICA) correlates to clinical features of plaque destabilization.

Methods—Endarterectomy specimens from 37 consecutive patients undergoing surgery for high-grade ICA stenosis were stained immunocytochemically for macrophages (CD68) and T cells (CD3). The staining was quantified by planimetry of immunostained areas (CD68) or counting individual cells (CD3). Clinical evidence of plaque instability was provided by the preoperative assessment of recent ischemic symptoms attributable to the stenosis and of the occurrence of cerebral microembolism in transcranial Doppler ultrasound monitoring of the ipsilateral middle cerebral artery.

Results—The percentage of macrophage-rich areas and number of T cells per mm² section area were larger in recently symptomatic patients than in asymptomatic patients (macrophages: 18±10% versus 11±4%, P=0.005; T cells: 71.2±34.4 versus 40.5±31.4 mm², P=0.0005). The presence of microembolism was associated with an increase in macrophage-rich areas (P=0.011). Macrophage (19±10% versus 9±3%, P=0.0009) and T cell (71.5±39.0 versus 46.4±22 mm², P=0.045) infiltration were more pronounced in predominantly atheromatous than in fibrous plaques, but did not correlate significantly to the presence of surface ulceration or luminal thrombosis.

Conclusions—Our data suggest a role of plaque-infiltrating macrophages and T cells in the clinical destabilization of high-grade ICA stenoses. Inflammatory mechanisms may be a therapeutic target in patients with symptomatic ICA disease. (Stroke. 1998;29:1625-1630.)

Key Words: atherosclerosis ■ carotid arteries ■ cerebrovascular disorders ■ immunohistochemistry ■ leukocytes

There is increasing evidence that inflammatory processes play a central role in the pathogenesis of atherosclerosis.1 Atherosclerotic plaques exhibit significant infiltration by activated macrophages, T cells, and mast cells.2-5 Inflammatory cells release matrix-degrading enzymes and thrombogenic substances that may provoke plaque disruption and local thrombosis.6-9 Thereby, the local inflammatory process may be critically responsible for plaque destabilization manifesting clinically as acute ischemic syndromes such as unstable angina, myocardial infarction, and stroke.10

Arterio-arterial thromboembolism from extracranial stenoses of the internal carotid artery (ICA) is an important pathogenetic mechanism of ischemic stroke.11,12 However, even high-grade ICA stenoses (≥70% luminal narrowing) carry a highly variable annual risk of stroke that can be as high as 13% following a recent occurrence of transient cerebral or retinal ischemia or as low as 1% to 2% in clinically asymptomatic patients.13-15 In many patients with high-grade ICA stenosis, long-term transcranial Doppler ultrasonography (TCD) can reveal clinically silent formed-element microemboli passing through the ipsilateral middle cerebral artery.16 The rate of microemboli is higher in recently symptomatic than in asymptomatic patients,17 predicts the occurrence of future ischemic symptoms,18 and declines after carotid endarterectomy.19,20 This indicates that cerebral microembolism reflects a pathogenically relevant process located at the ICA atheroma and can provide reliable paraclinical evidence of “unstable ICA disease.”21,22

In the present study, we performed a quantitative immunocytochemical analysis of inflammatory infiltration in endarterectomy specimens from 37 consecutive patients undergoing surgery for high-grade ICA stenosis, and asked whether the extent of inflammation correlates to plaque instability as evidenced clinically by the occurrence of ischemic symptoms and the rate of cerebral microemboli.

Subjects and Methods

Patients
This prospective study included 37 consecutive surgical inpatients (13 women, 24 men; age range, 41 to 75 years; median, 60 years) enlisted to undergo carotid endarterectomy for extracranial high-grade ICA stenosis (≥70% luminal narrowing).14,15,23 The degree of luminal narrowing was determined by intra-arterial cerebral angiography using the criteria of the North American Symptomatic Carotid
Endarterectomy Trial (NASCET). All patients had not used antiplatelet drugs or oral anticoagulants for more than 5 days. The study was approved by the local ethics review committee and performed in accordance with institutional guidelines. Informed consent was obtained from all patients before each examination. Thirty-two of the patients were part of a study population that was the subject of a recently published pathoanatomic study of carotid endarterectomy plaques. For 7 of the original 39 patients of that study, only insufficient material for immunohistochemical analysis was still available. Therefore, additional 5 consecutive patients were included in the present study.

**TCD Monitoring**

All 37 patients received long-term TCD signal recording of the middle cerebral artery ipsilateral to the high-grade ICA stenosis for at least 1 hour as described in detail elsewhere. The audible TCD analog output signal was recorded digitally on tape (20 kHz sampling rate) for further off-line analyses and blinded rating. The “energy” (e) of a microembolic signal (MES) was calculated using the following formula: 

\[ e = 20 \log \text{(embolic signal power/background power)} \] 

where “embolic signal power” was the average of 4 fast Fourier transformation lines withing the MES and “background signal power” was the average of 2 Fourier transformation (FFT; 128 points, 75% overlap) lines including the MES. The agreement between 2 blinded observers for quantifying inflammatory infiltration in all specimens examined, although interindividually to a highly variable degree. Overall, macrophage and T-cell infiltration occurred coincidentally, and was below for statistical analysis).

**Statistical Analysis**

The agreement between 2 blinded observers for quantifying inflammatory plaque infiltration was calculated from the independent analysis of n=67 sections for T cells and n=102 sections for macrophages according to Bland and Altman. The relationship between inflammatory infiltration and clinical features of plaque destabilization and between inflammatory infiltration and pathoanatomic features of the plaques was examined using the Mann-Whitney U test. Because we performed 2 statistical tests for each analysis, values of \( P<0.025 \) (0.05/2) were considered to indicate statistically significant findings (a adjustment according to modified Bonferroni procedure). The proportions of asymptomatic and symptomatic patients in the 2 microemboli groups were compared with the use of \( \chi^2 \) statistics.

**Results**

Using NASCET criteria, 21 of the 37 consecutive patients were defined as “symptomatic” based on a history of recent (less than 121 days before enlistment) occurrence of transient retinal or cerebral symptoms or minor ischemic stroke attributable to the high-grade ICA lesion. “Asymptomatic” patients (n=16) were defined as those who had a history of no or only remote (more than 120 days) ischemic symptoms. Between both groups there were no significant differences with respect to age and sex distribution, the degree of stenosis, and the size of the specimen (total section area) obtained by endarterectomy (Table 1).

Our immunohistochemical analysis revealed significant inflammatory infiltration in all specimens examined, although interindividually to a highly variable degree. Overall, macrophage and T-cell infiltration occurred coincidentally, and was

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**TABLE 1. Clinical Features of Symptomatic and Asymptomatic Patients with High-Grade ICA Stenosis**

<table>
<thead>
<tr>
<th></th>
<th>Asymptomatic</th>
<th>Symptomatic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>63</td>
<td>61</td>
</tr>
<tr>
<td>Sex, % Male</td>
<td>69</td>
<td>62</td>
</tr>
<tr>
<td>Luminal Narrowing, % (mean)</td>
<td>70–95 (84.6)</td>
<td>70–95 (82.5)</td>
</tr>
<tr>
<td>Total Section Area, mm² (mean)</td>
<td>105–347 (199.1)</td>
<td>83–393 (206.7)</td>
</tr>
<tr>
<td>Microemboli Count†</td>
<td>12</td>
<td>1</td>
</tr>
</tbody>
</table>

*Mann-Whitney U test: \( P=0.93 \).

†\( \chi^2 \) test: \( P=0.001 \), df=2.
most prominent in the fibrous cap overlying the atheromatous core of the lesions, especially in the immediate vicinity of the atheromatous core of the lesion. Scale bar = 50 μm.

With respect to the distribution of the cellular infiltrate, no major differences between symptomatic/asymptomatic or microemboli-positive/negative patients were apparent. Our subsequent quantitative analysis therefore focused on the overall macrophage and T-cell content of the plaques. Statistical analysis revealed that the percentage of macrophage-rich areas and the number of T cells per mm² section area were significantly greater in recently symptomatic than in asymptomatic patients (Table 2). In addition, macrophage infiltration was more pronounced in microemboli-positive than in microemboli-negative patients, and for T cells we found a clear trend (Table 3).

In 32 patients, both immunohistological and pathoanatomic data were available for comparative analysis. Macrophage and T-cell infiltration were significantly more pronounced in predominantly atheromatous (ie, lipid rich) than in fibrous plaques (Table 4). In contrast, no significant correlation was found between the extent of inflammatory infiltration and the presence of plaque ulceration or lumen thrombus.

**Discussion**

In the present study we performed a quantitative immunocytochemical analysis of macrophage and T-cell infiltration in endarterectomy specimens of 37 patients undergoing surgery for high-grade ICA stenosis. Plaque destabilization was evidenced clinically by the preoperative occurrence of ischemic symptoms and cerebral microembolism in the territory downstream of the stenosis. As the main finding we found a significant association of inflammation with the occurrence of ischemic symptoms and cerebral microemboli. Thus, in line with findings in coronary artery disease, our data suggest an important role of inflammation in the destabilization of advanced carotid artery plaques.

Since we found both T cells and macrophages to be more abundant in unstable compared with stable ICA plaques, both cell types may be involved in the process of plaque destabilization. In fact, a large body of evidence suggests that a complex interplay between T cells and macrophages is critical for the initiation and progression of atherosclerotic lesions. In atherosclerotic plaques, T cells express surface molecules and cytokines indicative of antigen-specific activation. In line with a proposed role of oxidized lipoproteins as local stimuli of a T-cell–mediated immune response, inflammatory infiltration was more pronounced in atheromatous, ie, lipid-rich, than in fibrous plaques. T-cell–derived cytokines like interferon-γ activate macrophages that in turn release matrix-degrading metalloproteinases or prothrombotic molecules like tissue factor. In coronary artery disease, the ensuing plaque rupture and luminal thrombosis are currently regarded as critical events in plaque destabilization manifesting clinically as unstable angina or myocardial infarction.

In light of this pathogenetic concept, it was surprising to find an association of inflammation with clinical signs but...
not with the putative morphological equivalents of plaque instability. However, although a statistically significant relationship was lacking, macrophage infiltration showed a trend toward higher values in ulcerated plaques. The lack of a significant correlation may therefore result from the relatively small sample size of our present study. On the other hand, even in coronary arteries evidence supporting a direct causal relationship between inflammation and the induction of plaque rupture and lumen thrombosis is still circumstantial. Current data are mainly derived from the autopsy study of patients with acute occlusive coronary thrombosis and subsequent fatal myocardial infarction. In this context, van der Wal et al.31 demonstrated local accumulations of macrophages and T cells at sites of intimal rupture or erosion. However, this study did not include control groups without clinical and/or morphological evidence of plaque instability. In a study based on atherectomy specimens from patients with both stable and unstable coronary syndromes, Moreno et al.30 found a correlation between the plaque content of macrophages and the occurrence of unstable angina, but did not further address the relationship between inflammation and patho-anatomic features of plaque destabilization. Recent patho-anatomic studies of high-grade carotid stenoses showed a strong association of plaque ulceration and lumen thrombosis with cerebral microembolism21 or clinical symp-
toms,40,41 and described local accumulations of macrophages and T cells at sites of plaque rupture.42 However, they did not conclusively correlate inflammation to clinical instability. To avoid any bias introduced by preselecting “areas of interest,” we focused our present analysis on the total plaque content of inflammatory cells rather than analyzing inflammation at sites of rupture or the most severe stenosis as done by Carr et al.42 Therefore, the influence of a particular distribution of inflammation was not assessed in our present study, but should be the subject of further analyses in larger patient populations.

In the present study, macrophage and T-cell densities both exhibited some overlap between clinically stable and unstable patients. Thus, we found single clinically unstable patients with relatively sparsely infiltrated plaques and vice versa. This suggests that multiple pathogenic factors contribute to the clinical manifestation of high-grade ICA stenosis. A recent autopsy study of sudden coronary death cases showed that a considerable proportion of acute coronary thromboses developed over only superficially eroded coronary lesions that exhibited significantly less inflammation than deeply ruptured plaques.43 With respect to our present findings, the question remains whether the pathogenic mechanism of plaque destabilization differs between strongly inflamed plaques and apparently “non-inflamed” lesions. Furthermore, it remains to be deter-

### TABLE 3. Relationship Between Preoperative Microemboli Count and ICA Plaque Infiltration for T Cells and Macrophages

<table>
<thead>
<tr>
<th>Microemboli Count</th>
<th>Percentage of macrophage-rich areas</th>
<th>Number of T cells per mm² section area</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 h⁻¹ (n=13)</td>
<td>11±5 (6–23)</td>
<td>45.1±27.1 (11–89)</td>
</tr>
<tr>
<td>&gt;0 h⁻¹ (n=24)</td>
<td>17±10 (7–44)</td>
<td>66.9±35.6 (12–158)</td>
</tr>
<tr>
<td>P*</td>
<td>0.011†</td>
<td>0.061</td>
</tr>
</tbody>
</table>

Values are mean±SD (range).
*Mann-Whitney U test.
†Significant after α adjustment (see “Methods”).

### TABLE 4. Relationship Between Pathoanatomic Plaque Features and ICA Plaque Infiltration for Macrophages and T Cells

<table>
<thead>
<tr>
<th>Plaque ulceration</th>
<th>n</th>
<th>Percentage of Macrophage-Rich Areas</th>
<th>P*</th>
<th>No. of T Cells per mm² section area</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absent</td>
<td>19</td>
<td>13±6</td>
<td>0.14</td>
<td>57.5±32.9</td>
<td>0.64</td>
</tr>
<tr>
<td>Present</td>
<td>13</td>
<td>18±11</td>
<td></td>
<td>64.3±39.3</td>
<td></td>
</tr>
<tr>
<td>Lumen thrombus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Absent</td>
<td>12</td>
<td>13±7</td>
<td>0.45</td>
<td>58.1±31.1</td>
<td>0.97</td>
</tr>
<tr>
<td>Present</td>
<td>20</td>
<td>16±10</td>
<td></td>
<td>61.5±37.8</td>
<td></td>
</tr>
<tr>
<td>Plaque composition</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predominantly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fibrous</td>
<td>17</td>
<td>9±3</td>
<td>0.0009†</td>
<td>46.4±22.2</td>
<td></td>
</tr>
<tr>
<td>Predominantly</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atheromatous</td>
<td>15</td>
<td>19±10</td>
<td></td>
<td>71.5±39.0</td>
<td>0.045</td>
</tr>
</tbody>
</table>

Values are mean±SD. n=32 specimens.
*Mann-Whitney U test.
†Significant after α adjustment (see “Methods”).
mined whether highly inflamed but clinically stable plaques carry an increased risk of future destabilization. Investigations based on endarterectomy specimens represent a “one-time-point” kind of analysis that necessarily disregards the dynamic nature of the underlying pathogenic processes. In particular, such studies leave the open question of whether inflammatory infiltration in fact precedes rather than follows plaque disruption, for example as part of a healing process reestablishing vessel integrity. The easy accessibility of the carotid artery might help to develop noninvasive diagnostic means that allow the direct monitoring of inflammatory activity in vivo. This provides the basis for prospective studies addressing the prognostic significance and therapeutic implications of inflammation in advanced atherosclerotic lesions.

Acknowledgments

We thank Prof W. Sandmann for providing the endarterectomy specimens on which our study was based. This study was supported in part by the Deutsche Forschungsgemeinschaft (Si 370/4–1). Drs. Steinmetz and Stoll hold Hermann-and-Lilly Schilling professorships.

References

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Stroke. 1998;29:1625-1630
doi: 10.1161/01.STR.29.8.1625

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0039-2499. Online ISSN: 1524-4628

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