Interleukin 23 Levels Are Increased in Carotid Atherosclerosis
Possible Role for the Interleukin 23/Interleukin 17 Axis

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Background and Purpose—Interleukin (IL)-23 is a cytokine in the IL-12 family, mainly produced by antigen-presenting cells with a central role in inflammation. We hypothesize that IL-23 is also important in atherogenesis and investigate this in a population with carotid atherosclerosis.

Methods—Plasma levels of IL-23 were measured in patients with carotid artery stenosis and in healthy controls. The mRNA levels of IL-23 and its receptor, IL-23R, were measured in atherosclerotic plaques, nonatherosclerotic vessels, peripheral blood mononuclear cells, and plasmacytoid dendritic cells.

Results—Our findings were as follows: (1) patients with carotid atherosclerosis (n=177) had significantly raised plasma levels of IL-23 when compared with healthy controls (n=24) with particularly high levels in those with the most recent symptoms. (2) mRNA levels of IL-23 and IL-23R were markedly increased in carotid plaques (n=68) when compared with nonatherosclerotic vessels (n=8–10). Immunostaining showed colocalization to plaque macrophages. (3) Patients with carotid atherosclerosis had increased mRNA levels of both IL-23 and IL-23R in plasmacytoid dendritic cells, but not in peripheral blood mononuclear cells. (4) IL-23 increased IL-17 release in monocytes and particularly in peripheral blood mononuclear cells from patients with carotid atherosclerosis, but not in cells from healthy controls. (5) IL-23 gave a prominent tumor necrosis factor release in monocytes from patients with carotid atherosclerosis but not in cells from healthy controls. (6) High plasma levels of IL-23 were associated with increased mortality during follow-up.

Conclusions—we have shown an association between IL-23 and disease progression in patients with carotid atherosclerosis, potentially involving IL-17-related mechanisms. (Stroke. 2015;46:793-799. DOI: 10.1161/STROKEAHA.114.006516.)

Key Words: atherosclerosis ■ carotid stenosis ■ inflammation ■ interleukins

Stroke is one of the major causes of death and disability worldwide. Approximately 85% of all strokes are ischemic, and 20% to 30% of these are caused by carotid atherosclerosis. Moderate- to high-grade carotid stenosis can be detected in 1% to 3% of adults, and the incidence increases with age.1-3 Along with the degree of stenosis, inflammation and plaque composition are important in predicting the risk of clinical symptoms.4 The atherosclerotic plaque is composed of infiltrating inflammatory cells (eg, monocytes/macrophages, T cells, and dendritic cells [DCs]), smooth muscle cells, and lipids. Plaques prone to rupture have thin fibrous caps and high-grade inflammation. The balance between pro- and anti-inflammatory mediators is therefore of major importance for the fate of the plaque and for the occurrence of adverse events.5,6
Interleukin (IL)-23 belongs to the IL-12 family of cytokines and consists of the 2 subunits p19 and p40. IL-23 is mainly produced by macrophages and DCs, and through its interaction with the IL-23 receptor (IL-23R), composed of the IL-12Rβ1 unit and the specific IL-23R unit, plays a central role in inflammation including the induction of Th17 cells. IL-23 has been connected with various inflammatory disorders, such as inflammatory bowel diseases, rheumatoid arthritis, and psoriasis. IL-23 has also been implicated in atherogenesis. Thus, patients with peripheral arterial disease have raised serum levels of IL-23, and increased IL-23 expression is seen in human carotid lesions. Zhang et al showed that carriers of a polymorphism in the IL-23R gene, which also gave increased expression of IL-23R in peripheral blood mononuclear cells (PBMCs), had increased risk of coronary artery disease. However, decreased IL-23 in PBMCs from patients with coronary artery disease has also been reported. In a mouse model of ischemic stroke, IL-23 levels were upregulated in both the circulation and in the brain. However, the role for IL-23 in atherosclerosis is still unclear.

In this study, we examined the expression of IL-23 in patients with carotid atherosclerosis both systemically and within the atherosclerotic plaque as well as its relation to adverse events during follow-up. We also examined the effect of IL-23 in PBMCs and monocytes from patients with carotid atherosclerosis and healthy controls.

Materials and Methods

A description of tissue sampling from carotid plaque and nonatherosclerotic vessels, immunohistochemistry, immunofluorescence, isolation of PBMCs, monocytes and plasmacytoid DCs (pDCs), stimulation of cells, and real-time quantitative polymerase chain reaction is given in the online-only Data Supplement.

Patients and Control Subjects

One hundred and seventy-seven patients with moderate (50%–69%) or severe (≥70%) internal carotid artery stenosis, treated with carotid endarterectomy (n=147), carotid artery stenting (n=10), or conservatively (n=20), were recruited at Department of Neurology, Oslo University Hospital, Rikshospitalet (Table I in the online-only Data Supplement). The patients were classified into 2 groups according to their symptoms: (1) 69 patients (39%) had a stroke, transient ischemic attack, or amaurosis fugax ipsilateral to the stenotic internal carotid artery in the previous 2 months, and (2) 108 patients (61%) had symptoms >2 months ago or no relevant symptoms detected during clinical examinations of patients with coronary artery disease or peripheral artery disease. The groups are further denoted as symptomatic and asymptomatic, respectively. Carotid stenoses were diagnosed and classified by precerebral color duplex and computed tomographic angiography. Ultrasound plaque appearance in terms of echogenicity was classified according to consensus criteria. Exclusion criteria were concomitant disease that could influence inflammation, such as infections, connective tissue disease, or malignancies, heart failure, and liver or kidney disease. For comparison, blood samples were collected from 24 healthy control subjects recruited from the same area of Norway as the patients. All controls were apparently healthy individuals as assessed by disease history, clinical examination, and normal levels of C-reactive protein. The protocols were approved by the Regional Committee for Medical and Research Ethics, South-East, Norway, ref S-0923a 2009/6065. Signed informed consent for participation in the study was obtained from all individuals.

Blood Sampling Protocol

Venipuncture of a forearm vein was performed within 2 days before carotid endarterectomy/carotid artery stenting with minimal stasis. Blood was drawn into pyrogen-free tubes with ethylenediaminetetraacetic acid as anticoagulant. The tubes were immediately immersed in melting ice and centrifuged within 30 minutes at 2500 g for 20 minutes to obtain platelet-poor plasma. All samples were stored at −80°C.

Cytokine Measurements

Concentrations of IL-23 in plasma and IL-17A/F (denoted IL-17) and tumor necrosis factor (TNF) in PBMC (IL-17) and monocyte (TNF) supernatants were analyzed by enzyme immunoassay (R&D Systems). IL-17 levels in monocyte supernatants and plasma were measured by Bioplex technology (BioRad Laboratories, Hercules, CA).

Statistical Analysis

For comparison of 2 groups of individuals, the Mann–Whitney U test was used. The χ² test was used for analyzing contingency data. In the intravital studies, Wilcoxon signed-rank test and Mann–Whitney U test were used for paired and unpaired data, respectively. Coefficient of correlation was calculated by the Pearson or Spearman rank test depending on the distribution of data. Kaplan–Meier analysis with log-rank test was performed to compare the number of events in relation to dichotomized IL-23 levels. IL-23 was dichotomized according to the median value in the patient group as a whole. The importance of IL-23 as a risk factor for all-cause mortality was further investigated by multivariable Cox regression including variables that were imbalanced (P<0.05) between survivors and nonsurvivors (ie, age and total leukocyte counts). Probability values (2-sided) were considered significant at P<0.05. All calculations were performed with SPSS for Windows statistical software (Version 18.0; SPSS Inc, Chicago, IL) or Graphpad Prism (Version 6.0; GraphPad Software, Inc., San Diego, CA).

Results

Increased Plasma Levels of IL-23 in Patients With Carotid Atherosclerosis

Patients with carotid atherosclerosis (n=177) had markedly raised plasma levels of IL-23 compared with healthy controls (n=24) (median: 295 versus 94 pg/mL; P<0.01), with particularly high levels in patients with the most recent symptoms (ie, symptoms within the last 2 months, n=69; median and [range]: 430 [1–8779] pg/mL; P<0.001 versus controls 94 [3–391] pg/mL; Figure 1). Also when dividing the patients in other subgroups (symptoms <1 months ago [n=55]; symptoms 2–6 months ago [n=36]; symptoms >6 months ago or no symptoms [n=72]), the patients still had higher levels of IL-23 than healthy controls (IL-23, median and [range]: 438 [1–8779] pg/mL; P<0.001 versus controls; 345 [1–8638] pg/mL; P=0.007 versus controls and 173 [1–6268] pg/mL; P=0.05 versus controls, respectively). Thus, regardless of division, all subgroups of patients had higher levels of IL-23 than controls.

Of all baseline variables outlined in Table I in the online-only Data Supplement, including C-reactive protein, only IL-23 and use of clopidogrel were significantly associated with recent ischemic symptoms (ie, within 2 months, n=69) when compared with asymptomatic patients (ie, symptoms ≥2 months ago or no relevant symptoms, n=108). In the patient group as a whole, IL-23 levels were moderately correlated with age (r=0.2; P<0.05) and total leukocyte count (r=0.17; P<0.05). Although the patients were older than the controls.
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Plasma levels of interleukin (IL) 23 in patients with carotid atherosclerosis were significantly higher than in healthy controls. Plasma levels of IL-23 were measured by enzyme immunoassay in patients with carotid atherosclerosis (n=177) compared with levels in healthy controls (n=24). The patients were classified into 2 groups according to their symptoms: (1) 69 patients had a stroke, transient ischemic attack, or amaurosis fugax ipsilateral to the stenotic internal carotid artery in the previous 2 months (symptomatic patients), and (2) 108 patients had symptoms >2 months ago or no relevant symptoms (asymptomatic patients). Data are presented as median with interquartile range. *P<0.05, **P<0.001 versus controls and #P<0.05 versus asymptomatic patients.

Gene expression of IL-23 and IL-23R was quantified by quantitative polymerase chain reaction in plaques from patients with carotid atherosclerosis (n=68) and in nonatherosclerotic vessels obtained from organ donors (n=8–10, common iliac artery from 8 cardiac donors and 2 donors of kidney and liver). Levels of expression are related to reference gene (β-actin) and normalized to expression in the control samples. Data are presented as mean±SEM. ***P<0.001 versus controls.

Increased Expression of IL-23 and IL-23R in Carotid Atherosclerotic Plaques

Gene expression of IL-23 and IL-23R was quantified by quantitative polymerase chain reaction in plaques from patients with carotid atherosclerosis (n=68) and in nonatherosclerotic control vessels (common iliac artery of organ donors, n=8–10). There were markedly increased levels of both transcripts in plaques compared with control samples with particularly increased levels of IL-23R (18-fold increase; Figure 2).

Increased Expression of IL-23 and IL-23R in pDCs From Patients With Carotid Atherosclerosis

DCs are an important cellular source of IL-23, and this cytokine may in turn modulate the function of these cells. As shown in Figure 4, pDCs from patients with carotid atherosclerosis (n=10) had markedly increased mRNA levels of IL-23 (≈25-fold increase) and IL-23R (≈8-fold increase).
compared with pDCs from healthy controls (n=14). In contrast, expression levels of IL-23 and IL-23R in PBMCs, primarily containing lymphocytes and monocytes, showed no significant difference between patients (n=23–24) and controls (n=35–36).

**IL-23 Enhances IL-17 Release From PBMCs and Monocytes**

IL-23 is a known inducer of the Th17 cell subset, and hence the production of IL-17. To explore this interplay in relation to atherosclerosis, we stimulated PBMCs from patients with carotid atherosclerosis (n=9) and healthy controls (n=8) with IL-23 (100 ng/mL) alone or in combination with phytohaemagglutinin (50 μg/mL) for 48 hours. Although IL-23 had no effect in PBMCs from healthy controls, it significantly increased IL-17 release in cells from patients with carotid atherosclerosis both when given alone and in phytohaemagglutinin-stimulated cells (Figure 5A).

To further elucidate the IL-23/IL-17 axis, we isolated monocytes from patients with carotid atherosclerosis (n=6) and healthy controls (n=5–6). The cells were preactivated by TNF to mimic the situation within an inflammatory microenvironment, and thereafter stimulated with IL-23 (100 ng/mL) alone or in combination with lipopolysaccharide (10 ng/mL) for 24 hours. The release of IL-17 from monocytes was in general low, and in some subjects absent. However, IL-23 induced a modest but significant increase in IL-17 release from patient cells, but not from control cells (Figure 5B). Our findings could suggest an interaction between IL-23 and IL-17, but we found no correlation between IL-17 and IL-23 either within the lesion (r=−0.2; P=0.09) or in plasma (r=−0.02; P=0.8). In plasma, the lack of correlation could at least partly reflect that several of the samples were low or below the detection limit of the IL-17 assay.

**IL-23 Promotes TNF Release in Monocytes From Patients With Carotid Atherosclerosis**

To further examine the inflammatory potential of IL-23 in monocytes, we examined the release of TNF in these cells after stimulation with IL-23 with or without costimulation with lipopolysaccharide. These experiments show that IL-23 enhanced the lipopolysaccharide-stimulated release of TNF in patients but not in controls (Figure 5C). Our data show that IL-23 has potent inflammatory effects in monocytes from patients with carotid atherosclerosis, beyond that of IL-17 release.

**High Plasma Levels of IL-23 Are Associated With Mortality**

To further elucidate the relationship of IL-23 to carotid atherosclerosis, we finally examined the association of plasma IL-23 levels to adverse outcome in the present study population (n=177; Table I in the online-only Data Supplement). During follow-up (mean 3.5 years), 28 of the patients died. About half of the mortalities (n=13) were caused by cerebrovascular or cardiovascular events. High IL-23 levels in plasma (ie, >median 295 pg/mL) were significantly associated with increased mortality also when adjusting for variables that were imbalanced (P<0.05) between survivors and nonsurvivors (age and leukocyte count; Figure 6).

**Discussion**

Previous studies have shown increased expression of IL-23 in carotid lesions with particularly high levels in symptomatic patients. In this study, we extend these findings by showing that the increased expression of IL-23 in carotid plaques is accompanied by an enhanced expression of its receptor IL-23R, both primarily located to macrophages. These patients also had increased levels of IL-23 in plasma and markedly increased expression of IL-23/IL-23R in pDCs. High plasma levels of IL-23 were associated with recent symptoms and with increased mortality during follow-up, underscoring the association between IL-23 and carotid atherosclerosis.

DCs are key modulators of immunity, pivotal in promoting innate and adaptive immune responses. pDCs have been
interferon-α-related mechanisms. In this study, we have shown that patients with carotid atherosclerosis have markedly enhanced expression of IL-23/IL-23R in pDCs, pointing to a role of IL-23 in relation to pDC activity in these patients.

To elucidate the effect of the increased IL-23/IL-23R levels in patients with carotid atherosclerosis, we stimulated PBMCs from patients and controls with IL-23. In vitro stimulation of PBMCs gives the opportunity to study the interplay between different immune cells and

![Figure 5.](image1.png)

**Figure 5.** Effects of interleukin (IL) 23 on IL-17 release in peripheral blood mononuclear cells (PBMCs) and monocytes. PBMCs from patients with carotid atherosclerosis (n=9) and healthy controls (n=8) were stimulated with IL-23 (100 ng/mL), phytohaemagglutinin (PHA) (50 μg/mL), or a combination thereof for 48 hours. Tumor necrosis factor (TNF; 5 ng/mL)-preactivated monocytes (48 hours) from patients with atherosclerosis (n=6) and healthy controls (n=5–6) were further stimulated with IL-23 (100 ng/mL), lipopolysaccharide (LPS; 10 ng/mL), or a combination thereof for 24 hours. IL-17 (A) in supernatants was measured by Bioplex, and TNF (C) was measured by enzyme immunometric assay. Under the following conditions, the absolute values were different between patients and controls: PBMCs, IL-17 release: IL-23+PHA-stimulated cells (P<0.05); monocytes, TNF release: unstimulated and IL-23 stimulated (P<0.05), LPS and LPS+IL-23 stimulated (P<0.01). Data are presented as mean±SEM. *P<0.05 and **P<0.01 versus unstimulated cells, #P<0.05 versus PHA/LPS-stimulated cells.

![Figure 6.](image2.png)

**Figure 6.** High plasma levels of interleukin (IL) 23 are associated with increased mortality. Association between plasma levels of IL-23 and mortality in patients shown by a Kaplan–Meier curve with the cumulative incidence of all-cause mortality during the entire study (mean follow-up of 3.5 years) according to dichotomized IL-23 levels (cutoff median, 295 pg/mL; P<0.05). CI indicates confidence interval; and HR, hazard ratio.
could thereby mimic the in vivo situation of plaque inflammation. We found that IL-23 significantly increased IL-17 release from patients, but not from control PBMCs. Also, IL-23 further potentiated the phytohaemagglutinin-inducing effect on IL-17 release, pointing toward an acceleration of a preactivated inflammatory status in these patients. An IL-23-inducing effect on IL-17 release was also found in monocytes from patients with carotid atherosclerosis, but not in monocytes from healthy controls. However, the IL-17 response from monocytes was in general low suggesting that monocytes are not an important cellular source of IL-17 within the PBMCs. Whereas the IL-17-inducing effect of IL-23 in monocytes was rather low, IL-23 markedly increased TNF release in these cells when costimulated with lipopolysaccharide, and again, this was only seen in patient cells. Our data suggest an inflammatory potential of IL-23 in PBMCs and monocytes, and these data also underscore the importance of examining cells from both patients and healthy controls when studying functional properties of cells in relation to atherosclerosis.

The role of IL-17 in atherosclerosis has been debated, but our in vitro results may strengthen its position as a potential proatherogenic mediator. Thus, we suggest that the IL-23/IL-17 axis, well characterized in many other inflammatory conditions, also may play a role in atherosclerotic disease. Recently, however, IL-23 has also been shown to have important proatherogenic effects on macrophages, independently of IL-17. In our study, IL-23 significantly increased TNF release in patient monocytes, and we also found colocalization of IL-23/IL-23R and macrophages within the atherosclerotic lesion, supporting an interplay between monocytes/macrophages and IL-23 in atherosclerotic disease. In contrast to the increased expression of IL-23/IL-23R in carotid plaques, this was not seen in PBMCs from these patients, suggesting that the upregulation of IL-23/IL-23R in PBMC-related cells, such as macrophages, within the atherosclerotic lesion reflects processes within the plaque. However, the relationship between IL-23 and different immune cells in atherosclerosis needs further investigation.

This study has some limitations, such as a relatively low number of patients and controls, and the controls were not perfectly matched with the patient group. The lack of functional data from pDCs and T cells limits the effect of our data, and the mortality data should be interpreted with caution because of a low number of events. Moreover, the association between IL-23 and carotid atherosclerosis does not necessarily imply any causal relationship. Even so, our findings may suggest a potential proatherogenic role of IL-23, and this possibility should be further investigated in larger clinical studies as well as in more mechanistic studies, including studies in experimental atherosclerosis.

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We thank Ellen Lund Sagen at the Institute for Internal Medicine, Oslo University Hospital, and the transplantation surgeons at Oslo University Hospital for their contribution to this work.

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Disclosures
None.

References


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SUPPLEMENTAL MATERIAL

Interleukin 23 levels are increased in carotid atherosclerosis – a possible role for the IL-23/IL-17 axis
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Methods

Tissue sampling from carotid plaque and non-atherosclerotic vessels

Atherosclerotic carotid plaques were retrieved from patients during carotid endarterectomy (n=68) and rapidly frozen in liquid nitrogen. For comparison, non-atherosclerotic vessels were obtained from the common iliac artery of deceased organ donors (8 cardiac donors and 2 donors of kidney and liver). Control tissues were prepared and stored in the same way as carotid plaques.

Immunohistochemistry

Sections (5 µm) of paraffin embedded carotid atherosclerotic were treated with 0.5% H202, followed by high-temperature unmasking in citrate-buffer (pH 6), blocked with 0.5% bovine serum albumin (BSA) and then incubated with primary antibodies (anti-IL-23; LSBio, Seattle, USA and anti-IL-23 Receptor; Abcam, Cambridge, UK) for one hour at room temperature. After washing, the slides were incubated for 30 minutes with peroxidase-conjugated secondary antibodies (Impress-Vector, Vector laboratories, Burlingame, CA), rinsed and developed with chromogen for immunoperoxidase staining (DAB Plus, Vector laboratories) for 7 minutes. The sections were counterstained with Hematoxylin. Omission of the primary antibody served as a negative control.

Immunofluorescence

Paraffin-embedded sections (5 µm) of atherosclerotic carotid plaques were exposed to high-temperature unmasking (citrate-buffer, pH 6), blocked in 0.5% BSA and incubated over night at 4°C with anti-human IL-23R (AbCam), anti-human IL-23 (LSBio) and anti-human CD68 (DAKO, Glostrup, Denmark). The sections were counterstained with Alexa Fluor 568- and 488-conjugated IgG, respectively (all from Invitrogen, Eugene, OR). Nuclei were stained with diamidino-2-phenylindole (DAPI) (Slow Fade Gold antifade reagent, Invitrogen). Fluorescent images were obtained on a Nikon Eclipse E400 microscope with 400× magnification.

Isolation of PBMCs, monocytes and plasmacytoid DCs (pDCs)

PBMCs were isolated from heparinized blood from healthy controls and patients with symptomatic carotid atherosclerosis by Isopaque-Ficoll (Lymphoprep; Nycomed, Oslo, Norway) gradient centrifugation and (i) stored in liquid nitrogen as cell pellets or (ii) used for further isolation of monocytes and pDCs. Briefly, the B220 positive fraction was depleted with magnetic beads (Miltenyi Biotec, Bergish Gladbach, Germany) after which pDCs were isolated from the negative fraction using a BDCA-4 isolation kit (Miltenyi Biotec). The purity for BDCA-4⁺ pDCs, as assessed by flow cytometry (CD123high BDCA-2high) was 96%. The purified pDCs were immediately stored in liquid nitrogen and subjected to RNA isolation.
Monocytes were isolated from freshly isolated PBMC by plastic adherence as previously reported.  

**Stimulation of cells**

Freshly isolated PBMCs from patients with symptomatic carotid atherosclerosis (n=9) and healthy controls (n=8) were incubated in flat-bottomed 24-well microtitre plates (2×10⁶/mL, 500 μL/well; Costar, Sigma-Aldrich, St. Louis, MO) with RPMI 1640 medium (PAA Laboratories, Pasching, Austria) supplemented with 10% heat inactivated human AB⁺ serum (Invitrogen, Carlsbad, CA) and 1% Penicillin-Streptomycin (Sigma). The cells were stimulated with recombinant human IL-23 (100 ng/mL, R&D Systems, Minneapolis, MN) with and without phytohaemagglutinin (PHA, 50 μg/mL; Thermo Scientific, Waltham, MA). Cell-free supernatants were harvested after 48 hours and stored at −80°C. Primary monocytes from healthy controls (n=5-6) and patients with symptomatic carotid atherosclerosis (n=6) were stimulated for 48 hours with TNF (5 ng/mL, R&D Systems) to mimic the inflammatory microenvironment within an atherosclerotic lesion, and thereafter with IL-23 (100 ng/mL, R&D Systems), lipopolysaccharide (LPS) (0111:B4 from *Escherichia coli*; 10 ng/mL, Sigma) or a combination thereof for 24 hours. Cell supernatants were harvested and stored at −80°C. The doses of IL-23, PHA and LPS, and the incubation time were based on preceding dose-response experiments.

**Real-time quantitative PCR**

Total RNA was obtained from atherosclerotic plaques and non-atherosclerotic vessels and from pDCs and PBMCs with the use of RNeasy spin columns (QIAGEN, Hilden, Germany). All samples were subjected to DNase treatment (RQI DNase; Promega, Madison, WI) and stored at −80°C until further analysis. cDNA synthesis was performed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA). Gene expression was examined by real-time quantitative PCR (qPCR) 7500 Fast Real-Time PCR System, Applied Biosystems). mRNA detection of IL-23, IL-23R and reference gene cyclophilin-A in pDCs were assessed with SyberGreen primers. Sequence specific primers could be provided on request. Sequence specific TaqMan primers and probes were used for IL-23, IL-23R and β-actin mRNA detection in the plaques and PBMCs (Assay-ID: IL-23: Hs00900828_g1; IL-23R: Hs00332759_m1; β-actin: 4310881E, Applied Biosystems). The relative mRNA level of each transcript was calculated by the ΔΔCt-method.

**References**

Supplementary Figure I

The panels demonstrate positive staining of IL-23 and IL-23R in carotid atherosclerotic plaques. Both shown with an overview picture taken with the 10X objective and a zoom with the 40X objective in the same area. IL-23 as well as IL-23R are present in areas with inflammatory cells.
Supplementary Table I. Baseline variables in patient groups according to symptomatology (n=177) and in control group (n=24)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Symptoms &lt;2 months n=69</th>
<th>Symptoms&gt;2 months or asymptomatic n=108</th>
<th>Control group n=24</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male sex</td>
<td>65.2 (45)</td>
<td>63.9 (69)</td>
<td>62 (15)</td>
<td>0.8</td>
</tr>
<tr>
<td>Age (Years)**</td>
<td>66.2 (8.7)</td>
<td>66.7 (8.7)</td>
<td>57.5 (10.0)</td>
<td>0.9</td>
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<tr>
<td>Statin treatment, % (n)</td>
<td>87.0 (65)</td>
<td>84.3 (91)</td>
<td>0</td>
<td>0.6</td>
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<tr>
<td>Acetylsalisylic acid, % (n)</td>
<td>88.4 (61)</td>
<td>81.5 (88)</td>
<td>0</td>
<td>0.2</td>
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<tr>
<td>Dipyridamol, % (n)</td>
<td>10.1 (7)</td>
<td>15.7 (17)</td>
<td>0</td>
<td>0.2</td>
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<tr>
<td>Warfarin, % (n)</td>
<td>11.6 (8)</td>
<td>11.1 (12)</td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>Clopidogrel, % (n)</td>
<td>44.9 (31)</td>
<td>22.2 (24)</td>
<td>0</td>
<td>0.001</td>
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<tr>
<td>Hypertension</td>
<td></td>
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<td></td>
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<tr>
<td>Ipsilateral ischemia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>on cerebral MRI</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Echolucent plaque</td>
<td>36.2 (25)</td>
<td>27.8 (30)</td>
<td>-</td>
<td>0.3</td>
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<td>Smokers</td>
<td>56.2 (39)</td>
<td>50.9 (55)</td>
<td>3 (12.5)</td>
<td>0.5</td>
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<td>IL-23 pg/mL*</td>
<td>420 (1-8779)</td>
<td>239 (1-8638)</td>
<td>93.7</td>
<td>0.027</td>
</tr>
<tr>
<td>Degree of stenosis (%)*</td>
<td>85 (50-99)</td>
<td>80 (50-95)</td>
<td>-</td>
<td>0.2</td>
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<tr>
<td>Cholesterol (mM)**</td>
<td>4.4 (1.2)</td>
<td>4.3 (0.9)</td>
<td>6.1 (0.9)</td>
<td>0.6</td>
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<tr>
<td>HDL cholesterol (mM)**</td>
<td>1.3 (0.5)</td>
<td>1.3 (0.4)</td>
<td>1.8 (0.4)</td>
<td>0.5</td>
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<tr>
<td>LDL cholesterol (mM)**</td>
<td>2.6 (1.0)</td>
<td>2.6 (0.8)</td>
<td>3.9 (0.9)</td>
<td>0.3</td>
</tr>
<tr>
<td>Triglycerides (mM)**</td>
<td>1.5 (0.7)</td>
<td>1.6 (0.8)</td>
<td>1.0 (0.5)</td>
<td>0.5</td>
</tr>
<tr>
<td>CRP (mg/L)**</td>
<td>5.6 (6.9)</td>
<td>5.5 (7.5)</td>
<td>3.9 (3.7)</td>
<td>0.9</td>
</tr>
<tr>
<td>Leucocyte count (10^9/L)**</td>
<td>8.0 (1.9)</td>
<td>7.7 (2.1)</td>
<td>5.3 (1.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>HbA1c (%)**</td>
<td>5.9 (1.0)</td>
<td>6.1 (1.5)</td>
<td>5.6 (0.3)</td>
<td>0.4</td>
</tr>
<tr>
<td>BMI (per Kg/m²)**</td>
<td>25.9 (4.2)</td>
<td>26.1 (3.8)</td>
<td>24.5 (2.6)</td>
<td>0.9</td>
</tr>
<tr>
<td>Fibrinogen (g/L)**</td>
<td>4.0 (0.8)</td>
<td>4.0 (1.0)</td>
<td>-</td>
<td>0.9</td>
</tr>
<tr>
<td>Platelets (10^9/L)**</td>
<td>286.0 (77.1)</td>
<td>278.0 (68.8)</td>
<td>286.6 (79.8)</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Clinical symptoms include stroke, TIA or amaurosis fugax ipsilateral to the stenotic internal carotid artery. Numbers given as percentage (numbers) or * median (min-max) or ** mean (SD). BMI = body mass index. P-value indicates difference between symptomatic and asymptomatic group