Decrease in Circulating Myeloid Dendritic Cell Precursors in Coronary Artery Disease

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OBJECTIVES
We analyzed the frequency of myeloid dendritic cell (mDC) and plasmacytoid dendritic cell (pDC) precursors in blood of patients with coronary artery disease (CAD) and in atherosclerotic carotid plaques of patients with cerebrovascular disease (CVD).

BACKGROUND
Circulating DC precursors are reduced in several autoimmune diseases. Atherosclerosis has features of an autoimmune disease, such as the presence of autoantibodies or autoreactive T cells. Tissue-resident DCs were previously described in atheromata, and it is assumed that they are important for the activation of T cells against autoantigens there.

METHODS
Circulating mDC and pDC precursors were flow cytometrically detected in healthy controls (n = 19), CAD patients with stable (n = 20) and unstable angina pectoris (n = 19), and acute myocardial infarction (n = 17). In human carotid plaques (n = 65), mDC and pDC precursors were identified immunohistochemically.

RESULTS
Circulating mDC precursors were significantly reduced in patients with stable angina pectoris (0.19%, p = 0.04), unstable angina pectoris (0.16%, p = 0.004), and acute myocardial infarction (0.08%, p < 0.001) compared with control patients (0.22% of peripheral blood mononuclear cells). In contrast, pDC numbers were not significantly altered. Circulating mDC precursors inversely correlated with high-sensitivity C-reactive protein (r = −0.38, p = 0.001) or interleukin-6 (r = −0.42, p < 0.001). In contrast to pDC, significantly more mDC precursors were observed in vulnerable carotid plaques (24, 0.25 mm²; n = 31; p = 0.003) than in stable ones (6.4, 0.25 mm²; n = 34).

CONCLUSIONS
Similar to autoimmune diseases, circulating mDC precursors were significantly reduced in patients with CAD. The emergence of mDC precursors in vulnerable plaques suggests their recruitment into atheromata as a possible reason for their decrease in blood. In contrast, no significant association of circulating pDC precursors with atherosclerosis was observed.

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During recent years, several lines of evidence have indicated that inflammation and immune reactions are implicated in atherosclerosis and coronary artery disease (CAD). Atherosclerotic lesions contain large numbers of immune cells, such as macrophages, T cells, mast cells, and dendritic cells (DCs). Additionally, a systemic proinflammatory state with elevated serum levels of C-reactive protein (CRP) or interleukin (IL)-6 is detectable in patients with atherosclerotic vascular diseases (1,2). The importance of immune reactions for the progression of atherosclerosis was proven in gene-targeted mouse models with combined defects of immunity and apolipoprotein E (3,4). Additionally, a significantly increased cardiovascular risk was observed in patients with certain autoimmune disorders, such as rheumatoid arthritis (5) or systemic lupus erythematosus (6).

In previous studies, it was shown that inflammation in atherosclerosis is caused by (auto)immune reactions based on humoral and cellular immunity against certain (auto)antigens. One-fourth of all T cells cloned from human atherosclerotic plaques specifically recognized oxidized low-density lipoproteins (oxLDLs) (7,8). Furthermore, autoantigens, which play an important role in atherogenesis, are heat shock proteins (HSPs) (9) and β2-glycoprotein (GP)Ib (10). Serum titers of antibodies against oxLDL (11) or HSP 65 (12) are significantly associated with human carotid atherosclerosis. Chronic infections also were implicated in the pathogenesis of atherosclerosis. For example, high titers of antibodies against Chlamydia pneumonia were shown in patients with CAD (13), and Chlamydia pneumonia often is detectable in macrophages in atherosclerotic lesions (14).

In that context, it is of great importance that human and microbial HSP exhibit a high structural and antigenic similarity, which might cause the immune response against human HSP by cross immunity, accounting for the association between chronic infections and atherosclerosis (15). The presence of autoantigens in atherosclerosis raised the question whether the vaccination with autoantigens might alter the progression of atherosclerosis. Indeed, in animal models, it was shown that subcutaneous immunization against HSP 65 (16) or β2-GP Ib (17) significantly enhanced atherosclerosis, whereas induction of tolerance by oral administration of HSP 65 significantly reduced atherosclerosis (18). In contrast, the immunization against oxLDL remarkably reduced atherosclerosis (19). The reason for this discrepant response might be the induction of high-titer

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antibodies against oxLDL by the vaccination, which might increase the clearance of oxLDL from atherosclerotic lesions (20).

Dendritic cells play an important role as professional antigen-presenting cells in the immune system because of their unique ability to induce a primary immune response by activation of naive T cells (21). Two subtypes of DCs with different functions exist: 1) myeloid dendritic cells (mDCs), which express CD1c, CD11c, and CD33; secrete IL-12 after stimulation; and usually are activated after contact with bacterial products; 2) plasmacytoid dendritic cells (pDCs) with plasma cell-like appearance, which express CD123, the IL-3 receptor α-chain, respond to viral infections, and are the major source of interferon-α (22). In recent times, several specific antibodies against blood DC antigens (BDCA) were developed, enabling researchers to investigate the number of circulating mDC and pDC precursors in different immunological disorders by flow cytometry (23,24). So far, significant alterations of circulating DC precursors were described in autoimmune diseases such as lupus erythematosus (25,26), Sjögren's syndrome (27), sarcoidosis (28), allergic asthma bronchiale (29), as well as infectious diseases such as human immunodeficiency virus infection (30), chronic viral hepatitis (31–34), or tuberculosis (35). In lupus erythematosus, which is a prototype autoimmune disease, significant alterations of circulating DC precursors were determined in the present study the number of circulating DC precursors in patients with different stages of CAD. Additionally, mDC and pDC precursor frequency were analyzed in carotid atheromata to investigate their possible recruitment into atherosclerotic plaques.

**METHODS**

**Patients and controls.** Coronary angiography was performed in all studied patients and healthy controls by a standard technique. The patients included in our study had at least one diseased vessel (>75% stenosis). Exclusion criteria were noncardiac diseases that might interfere with our analysis: acute or chronic infections, malignancies, autoimmune diseases, hyperthyroidism, and medication with immunosuppressive agents. Blood samples were taken within 24 h after admission and in the case of myocardial infarction also after one week.

The patients were divided into three study groups. Patients with stable angina pectoris (SAP) had a long-term, stable effort angina that had lasted at least three months and a positive exercise test. Unstable angina pectoris (UAP) was defined as rest pain occurring within 48 h without a recent myocardial infarction (MI). In those patients, transient ST-T segment depression and T-wave inversion, but no significant elevation of cardiac enzymes, often were present. Criteria of an acute myocardial infarction (AMI) were typical angina associated with ST-segment elevations in electrocardiogram and at least three occurrences of elevated serum creatine kinase and troponin-I. The control group consisted of subjects who underwent a coronary angiogram for angina-like symptoms but for whom CAD was safely excluded by coronary angiogram. The study was approved by the institutional ethics committee of the University of Erlangen-Nuremberg, and informed consent was obtained from all patients and control patients.

**Serum analysis of proinflammatory markers.** The serum concentration of high-sensitivity CRP (hsCRP) was measured using an immunonephelometric assay on a BN II...
analyzer (Dade Behring, Marburg, Germany) according to the manufacturer’s instructions. The serum level of IL-6 was analyzed using an enzyme-linked immunosorbent assay kit (R&D Systems, Wiesbaden, Germany) according to the manufacturer’s instructions.

**Identification of DC precursors by fluorescence-activated cell sorting (FACS).** Cell surface antigens were analyzed immediately after sample submission. The following fluorescein isothiocyanate (FITC)-, phycoerythrin (PE)-, and PE-Cy5-conjugated monoclonal mouse anti-human antibodies were used: BDCA1-PE, BDCA2-PE (each from Miltenyi-Biotec, Bergisch Gladbach, Germany), CD14-PE-Cy5, CD18-FITC, CD19-PE-Cy5, CD86-FITC, HLA-DR-FITC (all from Caltag Laboratories, Hamburg, Germany), CD11a-FITC (BD Pharmingen, Heidelberg, Germany), and CD40-FITC (Southern Biotechnology, Birmingham, Alabama). Three color-staining and FACS analysis were performed according to the strategy, which is currently used in the Blood Dendritic Cell Enumeration Kit of Miltenyi-Biotec (23,24). In contrast to the procedure of the kit, mDC and pDC precursors were analyzed in separate samples and, in addition, the FITC channel was used to analyze the expression of several markers on mDC and pDC surface. In brief, in each sample, 200 μl of blood was mixed with 7 μl of anti-CD14-PE-Cy5, 7 μl of anti-CD19-PE-Cy5, 7 μl of anti-BDCA1-PE or anti-BDCA2-PE, 1 to 13 μl of a FITC-stained antibody, and 7 μl of propidium iodide (BD Pharmingen). Red blood cells were lysed using a FACS lysing solution (Becton Dickinson, Heidelberg, Germany). Cells were analyzed using a FACSCalibur flow cytometer with CellQuest software (Becton Dickinson). Appropriate isotype-matched immunoglobulins (all from Caltag, Burlingame, California) were used as negative controls.

Because circulating DC precursors comprise only 0.1% to 1% of peripheral blood mononuclear cells (PBMCs), a special gating strategy was used to analyze mDC and pDC numbers accurately (Fig. 1). For each sample, 3 × 10⁵ PBMCs were registered in region (R)1 defined by forward scatter and side scatter. In R2, granulocytes were excluded according to their high side scatter, and monocytes, lymphocytes, and dead cells were excluded according to their CD14, CD19, and propidium iodide staining, respectively. Circulating mDC and pDC precursors were detected from the remaining cell population according to their specific staining for BDCA-1 and BDCA-2 in R3a or R3b, respectively. The relative cell numbers of mDCs and pDCs were determined as a percentage of PBMC based on the ratio of gated cells in R3a and R3b to the gated cells in R1. The absolute cell numbers of mDCs and pDCs per micro-liter (cells/μl) were calculated based on the relative mDC and pDC numbers and the white blood cell count. To minimize any methodical variations, one blood sample of each patient was flow cytometrically analyzed in five independent experiments, and the median was calculated from the resulting data to describe mDC and pDC numbers.

![Figure 1. Gating strategy used for the identification of circulating myeloid dendritic cells and plasmacytoid dendritic cell precursors in peripheral blood by flow cytometry. R1 = gating of peripheral blood mononuclear cells according to their forward and side scatter. R2 = exclusion of granulocytes by side scatter, B lymphocytes by CD19-staining, monocytes by CD14-staining, and dead cells by propidium iodide-staining. R3a and R3b = detection of circulating mDC and pDC precursor as the result of their specific BDCA-1 and BDCA-2 staining, respectively.]
Additionally, CD11a, CD18, CD40, CD86, and HLA-DR expression on mDC and pDC precursors was analyzed.

**Histological characterization of atherosclerotic carotid plaques.** Atherosclerotic carotid specimens were decalcified in ethylene diamine tetracetic acid for 4 days. After paraffin embedding, serial cross sections (4 μm thick) were cut and mounted on glass slides. To analyze the plaque morphology, representative sections of each plaque were stained with trichrome. Digital images of atherosclerotic plaques were obtained by a CCD camera (Nikon DXM 1200, Düsseldorf, Germany). The size of the lipid core and of the total plaque area was analyzed by computer-aided planimetry (Lucia Software V. 4.21, Laboratory Imaging Ltd., Nuremberg, Germany). Fibrous cap thickness was measured at its narrowest site. As described previously (39), atherosclerotic carotid plaques were subdivided into the following groups: stable (lipid core <50%, fibrous cap >100 μM) versus. vulnerable (lipid core >50%, fibrous cap <100 μM).

**Immunohistochemical detection of DC precursors in atherosclerotic carotid plaques.** For immunohistochemical staining, the following monoclonal antibodies were used: anti-BDCA-1 for mDC precursors and anti-BDCA-2 for pDC precursors (each from Miltenyi Biotec), anti-fascin for tissue-resident DC (DakoCytomation, Hamburg, Germany), and anti-macrophage inflammatory protein (MIP)-3α (R&D Systems). Immunohistochemical stainings of serial sections were performed using the catalyzed signal amplification kit according to the manufacturer’s instructions (CSA System, DakoCytomation, Glostrup, Denmark). In brief, the specimens were treated with 3% hydrogen peroxide to quench endogenous peroxidase activity. Then, the specimens were blocked with serum-free protein, followed by incubation with the primary antibodies, and then by four sequential 15-min incubations with secondary biotinylated link antibodies, streptavidin-biotin-peroxidase complex, biotinyl tyramide (amplification reagent), and streptavidin peroxidase. Finally, brown staining was obtained by incubation with 3,3′-diaminobenzidine tetrahydrochloride. Negative controls were treated with irrelevant isotype-matched antibodies. Digital cell analyses were performed as previously described (39). Stained cells were identified with a CCD camera at a magnification of 150× in representative areas of 0.25 mm² of different plaque regions: fibrous cap, plaque shoulders, lipid core, and media. For each plaque, the mean cell number was calculated from the corresponding cell numbers of the different plaque regions.

**Statistical analysis.** Data were expressed as median with entire range; p < 0.05 was considered statistically significant. The nonparametric Mann–Whitney rank sum test was used to compare mDC and pDC numbers as well as the numerical clinical data between the study groups and the control group (1, SAP vs. CTL; 2, UAP vs. CTL; and 3, AMI vs. CTL). The categorical clinical data were compared in a similar manner by the Fisher exact test. The correlation between mDC or pDC and hsCRP or IL-6 values was analyzed by the nonparametric Spearman rank order test.

**RESULTS**

**Study populations.** Clinical characteristics of patients with CAD are reported in Table 1 (upper part). In our study, CAD patients with SAP (n = 20), with UAP (n = 19), and with AMI (n = 17) were compared with 19 healthy CTLs. No significant differences were observed with respect to age, gender, atherogenic risk factors, oral medication, and low-density lipoprotein/high-density lipoprotein cholesterol ratio. Patients with UAP and AMI were treated with intravenous heparin, aspirin, and nitroglycerin. Percutaneous interventions (PCIs) were performed more frequently in patients with AMI than those with UAP or SAP. Significantly increased levels of hsCRP and IL-6 were observed in patients with AMI (hsCRP: 21.8, 1.6 to 73.4 mg/l; IL-6: 20.1, 4.1 to 30.2 pg/ml, p < 0.001) compared with CTL (hsCRP: 2.1, 0.7 to 16.7 mg/l; IL-6: 4.4, 0.8 to 9.6 pg/ml), indicating a strong proinflammatory systemic state in those patients. Additionally, patients with AMI had significantly elevated leukocyte counts (12,600, 7,500 to 18,800/μl, p < 0.001) compared with CTL (6,700, 3,700 to 10,400/μl). As expected, significantly increased levels of creatine kinase and troponin-I were observed in patients with AMI. In contrast, the levels of hsCRP, IL-6, and leukocyte counts did not differ significantly between UAP or SAP compared with CTL. The values of other laboratory parameters were not significantly altered between all study groups and CTL (data not shown).

To analyze the emergence of DC precursors in atherosclerotic plaques, carotid specimens from patients with cerebrovascular disease were examined by immunohistochemistry. According to their plaque morphology, patients with cerebrovascular disease were subdivided into two groups: stable versus vulnerable (Table 1, lower part). Plaques of 34 patients with stable morphology and of 31 patients with vulnerable morphology were compared. There were no significant differences with respect to age, gender, atherogenic risk factors, or oral medication between both groups. In contrast, ischemic symptoms such as transient ischemic attack, stroke, or amaurosis occurred more often in patients with classified vulnerable (55%) than those with stable plaques (29%, p = 0.04).

**Decrease in circulating mDC precursors in CAD.** Circulating mDC and pDC precursors were identified according to their expression of BDCA-1 and BDCA-2, respectively, and the absence of the expression of other PBMC markers (23,24). In our FACS analyses, mDC numbers varied between 0.03% and 0.38% (1.6 to 36.6/μl) and pDC numbers between 0.01% and 0.29% (1.0 to 23.0/μl). These values were in the previously described range (24,34). Significantly lower percentages of circulating mDC precursors were observed in patients with SAP (0.19%, 0.03% to 0.37%, p = 0.04), UAP (0.16%, 0.06% to 0.31%, p = 0.004), and AMI (0.08%, 0.05% to 0.26%, p < 0.001) compared with CTL (0.22%, 0.09% to 0.38%) (Fig. 2A). In contrast, the percentages of pDC precursors were only slightly but not significantly reduced in patients with CAD.
In a similar way, the absolute numbers of mDC precursors were significantly lower in patients with SAP (11.3 /H9262 l, 1.6 /H9262 l to 36.6 /H9262 l, p/11005 0.03), UAP (10.3 /H9262 l, 2.6 /H9262 l to 19.8 /H9262 l, p/11005 0.01), and AMI (9.5 /H9262 l, 6.7 /H9262 l to 18.6 /H9262 l, p/11005 0.01) than in CTL (15.6 /H9262 l, 7.0 /H9262 l to 48.5 /H9262 l) (Fig. 2C).

This fact enabled us to exclude the possibility that a dilution of mDC precursors by an increase in another PBMC population might have caused their relative reduction in blood of patients with CAD. The absolute numbers of circulating pDC precursors were again only slightly, but not significantly reduced in patients with CAD (Fig. 2D).

Furthermore, we monitored the expression of the costimulatory molecules CD40 and CD86, as well as HLA-DR, and the adhesion molecules CD11a and CD18 on DC precursors. As previously reported (23,24), only a weak expression of CD40 or CD86 on circulating mDC or pDC precursors was observed (data not shown), which is explained by their immature state. In contrast, high amounts of HLA-DR, which is known to be constitutively expressed on DC precursors (23,24), were found on mDC and pDC precursors (data not shown). A high expression of CD11a and CD18 on both mDC and pDC precursors was observed. However, the expression of CD40, CD86, HLA-DR, CD11a, or CD18 on circulating mDC or pDC precursors did not differ significantly between the study groups and CTL group (data not shown).

<p>| Table 1. Clinical Data of Patients With CAD and CVD |</p>
<table>
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<th>Study Groups</th>
<th>CTL</th>
<th>SAP</th>
<th>UAP</th>
<th>AMI</th>
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<td></td>
</tr>
<tr>
<td>No. of patients</td>
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<td>19</td>
<td>17</td>
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<td>70</td>
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<td>55</td>
<td>74</td>
<td>41</td>
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<td>37</td>
<td>29</td>
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<tr>
<td>Hypertension</td>
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<td>75</td>
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<td>Smoking</td>
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<td>Family history of CAD</td>
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<td>25</td>
<td>42</td>
<td>35</td>
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<td>Current medication (%)</td>
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<tr>
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<td>50</td>
<td>37</td>
<td>29</td>
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<tr>
<td>Aspirin</td>
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<td>65</td>
<td>37</td>
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<tr>
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<tr>
<td>Percutaneous intervention (%)</td>
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<td>100</td>
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<td>6,200</td>
<td>6,500</td>
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<td>hsCRP (mg/l)</td>
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<td>2.2</td>
<td>6.2</td>
<td>21.8†</td>
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<tr>
<td>IL-6 (pg/ml)</td>
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<td>5.3</td>
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<td>Creatinine kinase (U/l)</td>
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<td>68.5</td>
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<td>1,818†</td>
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<td>Troponin-I (ng/ml)</td>
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<td>0.02</td>
<td>0.15</td>
<td>31.5†</td>
</tr>
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</table>

Plaque Morphology Stable Vulnerable

| Patients with CVD | | | | |
| No. of patients | 34 | 31 | | |
| Age (yrs) | 65 | 72 | | |
| Male (%) | 59 | 71 | | |
| Atherogenic risk factors (%) | | | | |
| Diabetes | 41 | | 39 | |
| Hypertension | 82 | | 81 | |
| Smoking | 27 | | 32 | |
| Hyperlipidemia | 68 | | 61 | |
| Current medication (%) | | | | |
| Statins | 24 | | 36 | |
| Aspirin | 62 | | 74 | |
| Acute ischemic symptoms (%) | 29 | | 55* | |

Upper part of the table shows clinical data of healthy controls (CTL), coronary artery disease (CAD) patients with stable angina pectoris (SAP) and unstable angina pectoris (UAP), and acute myocardial infarction (AMI). Values are reported as median or %. Each parameter of the patient groups (SAP, UAP, and AMI) was statistically compared pairwise with the control group (CTL). Significant differences between single values of a patient group and the corresponding one of the control group are indicated by daggers (‡p < 0.001). Other values, not marked with daggers, did not differ significantly between patient groups and controls. Lower part of the table shows clinical data of cerebrovascular disease (CVD) patients with histologically determined stable and vulnerable plaque morphology did not differ significantly, except for acute ischemic symptoms (*p < 0.05). Values are reported as median or %.

ACE = angiotensin-converting enzyme; HDL = high-density lipoprotein; hsCRP = high-sensitivity C-reactive protein; IL = interleukin; LDL = low-density lipoprotein.
Regarding the clinical data of the study patients, no significant association of the number of mDC or pDC precursors with age, gender, atherogenic risk factors, or oral medication was observed. When comparing SAP or UAP patients who underwent PCI with those without PCI, no significant changes in mDC or pDC numbers were detected, indicating that PCI itself had no influence on the decrease in mDC in our study patients (data not shown).

In patients with AMI, one week after the incident, we observed a decrease in hsCRP and IL-6 (Fig. 3A), simultaneously to a partial reconstitution of mDC percentages (Fig. 3B). However, no significant changes of pDC precursors were observed at the follow-up (Fig. 3C).

In our study patients, the number of mDC precursors significantly correlated inversely with hsCRP (Fig. 4A) or IL-6 (Fig. 4B). In contrast, no significant correlation between pDC precursors and hsCRP or IL-6 was detected (Figs. 4C and 4D).

**Increase in mDC precursors in vulnerable atherosclerotic carotid plaques.** To assess a possible recruitment of circu-
lating DC precursors into atherosclerotic plaques, immuno-
histochemical stainings of carotid atheromata were per-
formed. CD1a or CD1c molecules are specifically ex-
pressed on Langerhans cells, a rather immature precursor
form of mDC in the epidermis, and their expression was
reduced upon DC maturation (41,42). Thus, CD1a or
CD1c (=BDCA-1) represent excellent surface markers for
mDC precursors, and they are nowadays used for their
identification or isolation from peripheral blood (41). On pDC
precursors, BDCA-2 is specifically expressed, and it is down-
regulated upon DC maturation (23). Therefore, we used the
same antibodies for the immunohistochemical identification of
mDC and pDC precursors in atherosclerotic plaques as in
FACS analysis, BDCA-1 and BDCA-2, respectively. Tissue-
resident DCs were analyzed using an antibody against fascin as
previously described (39). Furthermore, we analyzed the ex-
pression of MIP-3α, the most potent chemokine for the
attraction of DC precursors into inflamed tissue (43).

Precursors of mDC were detected in all analyzed atheromata
(Fig. 5). The localization of mDC precursors corresponded well
to that of tissue-resident DC. The percentage of mDC precursors to
tissue-resident DC varied from 8% to 16%, suggesting a loss of
BDCA-1 expression after invasion into the tissue, as described for
mDC precursors in the epidermis (42). In contrast, only few pDC
precursors were found in 53 of all 65 atherosclerotic plaques. In
most of the analyzed atherosclerotic plaques, the expression of the
chemokine MIP-3α on macrophage-like cells was observed.
Comparing stable plaques of asymptomatic patients with vulner-
able plaques of patients with acute ischemic symptoms (examples
shown in Fig. 5), higher numbers of mDC precursors, tissue-
resident DC, and MIP-3α but not of pDC precursors seemed to
be present in vulnerable plaques. Indeed, using computer-aided
cell quantification, in vulnerable plaques compared with stable
ones, significantly higher numbers of mDC precursors (24 mm²,
0.8 mm² to 71.3/0.25 mm² vs. 6.4 mm², 0.2 mm² to 53.8/0.25
mm², \( p = 0.003 \); Fig. 6A), tissue-resident DC (129 mm², 30.4
mm² to 188.8/0.25 mm² vs. 81.2 mm², 14.2 mm² to 188/0.25
mm², \( p = 0.01 \); Fig. 6C), and MIP-3α (13.6 mm², 0 to 60.4/0.25
mm² vs. 1.8, 0 to 39.0/0.25 mm², \( p = 0.002 \)) (Fig. 6D) were
shown. In contrast, pDC numbers did not significantly differ
between stable and vulnerable plaques (Fig. 6B). Additionally, a
significant correlation (\( r = 0.28, p = 0.03 \)) between mDC
numbers and MIP-3α-expressing cells was observed (data not
shown), suggesting that MIP-3α attracts mDC precursors into
the atheromata.

**DISCUSSION**

In previous studies, tissue-resident DCs were detected in the
intima of susceptible arteries and in advanced athero-
sclerotic plaques (37,38). Particularly in vulnerable plaques, we previously described a high density of tissue-resident DC with frequent T-cell contacts, suggesting their contribution to plaque destabilization (39). Dendritic cells are able to regulate the immune response to foreign- as well as self-antigens, inducing either an immune response or tolerance (21). Former studies showed that immune reactions are involved in atherogenesis. Therefore, it is assumed that DCs might play a key role in those immune reactions in atherosclerosis.

Three distinct differentiation stages of DCs and their typical migratory route are well known (21,44). Dendritic cells are derived from a common CD34\(^+\) progenitor in the bone marrow and are released as circulating DC precursors into the blood. From the bloodstream, they enter various tissues and develop into tissue-resident, immature DC there, which are able to take up antigens. In response to inflammatory signals, their maturation is induced, enabling mature DCs to activate T cells after their migration into the lymph nodes. Dependent on the local cytokine environment, tissue-resident, immature DCs might alternatively arise from monocytes.

In our present study, we investigated whether the previously reported local increase in tissue-resident DCs in advanced atherosclerotic plaques might be associated with any changes of the frequency of circulating mDC or pDC precursors. We showed that the number of mDC precursors is significantly reduced in blood of patients with stable and unstable CAD compared with healthy patients. Additionally, patients with AMI exhibited a further temporary reduction in mDC precursors, which partially normalized after one week.

Similar to our observations in CAD, previous studies showed a decrease in circulating DC precursors in various autoimmune or infectious diseases. Dependent on the type of the immunological disease, either a predominant decrease in mDC precursors (Sjögren’s syndrome, tuberculosis, and asthma bronchiale) (27,29,35) or pDC precursors (lupus erythematosus, chronic hepatitis B and C) (25,26) was described. However, it is still yet unclear why different DC

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**Figure 5.** Immunohistochemical stainings of myeloid dendritic cell (mDC) precursors (BDCA-1), plasmacytoid dendritic cell (pDC) precursors (BDCA-2), tissue-resident DC (fascin), and the DC-specific chemokine MIP-3\(\alpha\) in the plaque shoulder (PS) and in the lipid core (LC) of carotid plaques (magnification, \(\times 150\)). (A) Representative example of an atherosclerotic plaque with stable morphology (thick fibrous cap, small lipid core) of an asymptomatic patient. (B) Representative example of an atherosclerotic plaque with a vulnerable morphology (thin fibrous cap, huge lipid core) of a patient with a transient ischemic attack (TIA). Note the remarkably higher frequency of mDC, tissue-resident DC, and MIP-3\(\alpha\) in the vulnerable plaque of the patient with TIA compared with the stable plaque of the asymptomatic patient. Only few pDC precursors were observed in both types of plaques.
precursor subtypes are altered in particular immunological diseases, and this fact might be associated with disease-specific immune mechanisms. In those former studies, possible mechanisms of the decrease in circulating DC precursors in inflammatory diseases were investigated, and it was shown that this decrease was accompanied with a local DC increase in the inflammatory lesions, indicating a recruitment of DC precursors from blood into the inflamed tissue (28,31,32,35). Indeed, in a rat model, a rapid DC precursor recruitment to inflammatory sites was shown after exposure to an inflammatory stimulus (45).

To elucidate the possibility of DC recruitment into atheromata in our patients with CAD, we analyzed the emergence of DC precursors in carotid plaques with vulnerable or stable morphology. Because the acquisition of complete coronary plaques is difficult and only possible postmortem, we used advanced carotid atheroma instead. In vulnerable plaques, we found significantly higher numbers of mDC precursors than in stable ones, indicating a recruitment of mDC precursors particularly at an unstable stage of atherosclerosis. The expression of MIP-3α, which is the most effective DC chemoattractant (43), was significantly greater in vulnerable than in stable carotid plaques, and it significantly correlated with mDC frequency. Thus, it is likely that the decrease in circulating mDC precursors in patients with CAD, particularly those with ACS, is caused by their recruitment into coronary plaques. However, we cannot exclude that other mechanisms, such as apoptosis or a reduced production of mDC precursors in bone marrow, might be responsible for their decrease. Because there was a strong decline of circulating mDC precursors in AMI patients with a partial recovery after one week, an additional invasion into ischemic myocardium might occur in the acute phase.

In concordance with our results, Weis et al. (46) showed that certain atherogenic determinants enhanced adhesion and transmigration of mDC across an endothelial cell monolayer. Additionally, in patients with coronary restenosis, a higher mDC number was observed in in-stent restenosis probes, suggesting that mDC are recruited into PCI-treated lesions and might be involved in restenosis (47). In our study, we did not observe any differences in circulating mDC precursors in patients treated with PCI compared with those without PCI. However, in contrast to the study of Skowasch et al. (47), most of our patients had an ACS.

Beyond local inflammation in atherosclerotic plaques, several inflammatory markers, which well correlate with the atherosclerotic burden, were identified in blood of patients with atherosclerotic diseases. Among those markers, hsCRP and IL-6 were proved to be most powerful in the prediction of future cardiovascular or cerebrovascular events (1,2). In the present study, we showed a strong inverse correlation between the decrease in circulating mDC precursors and
hsCRP or IL-6 levels. Thus, it is likely to assume that the decrease in circulating mDC precursors might provide another way to predict cardiovascular risk, particularly because mDC precursors were significantly reduced even at a stable stage of atherosclerosis. Therefore, we will investigate their prognostic value, especially in patients with asymptomatic CAD in future prospective studies.

In contrast to the significant decrease in circulating mDC precursors, circulating pDC precursors were only slightly reduced in patients with CAD. Additionally, we found only single pDC in carotid plaques, and their frequency was not associated with a particular plaque morphology. These divergent results about mDC and pDC emergence in atherosclerosis were somehow puzzling, because both DC subtypes arise from a common CD34+ progenitor form in the bone marrow. However, the following major differences between mDC and pDC, regarding their different migration pattern and function, might explain the divergent results observed in our study. Circulating mDC and pDC precursors exhibit a very similar pattern of chemokine receptors (48). However, in functional studies, it was clearly demonstrated that the migratory response of mDC and pDC to chemokines profoundly differed (48). Although mDCs mainly are attracted by proinflammatory chemokines such as MCP-1 or MIP-3a, pDCs respond much more to the homeostatic CXCL-12, which directs them into lymph nodes (48). In atherosclerosis, immune competent cells are mainly recruited into inflammatory infiltrates of atherosclerotic plaques and not so much into perivascular lymphatic tissue, explaining the higher alterations of circulating mDC precursors in contrast to circulating pDC precursors.

The results of the present study suggest a recruitment of circulating mDC precursors into unstable atheroma and their crucial involvement in the (auto)immune mechanisms in atherosclerosis. Recent studies described a connection between DC and atherosclerosis-relevant (auto)antigens. In vitro studies, it was demonstrated that oxLDL promotes the maturation of mDCs, enabling them to induce T-cell activation (49, 50). In addition, we recently showed the clustering of mDC particularly in lipid-rich areas of advanced atherosclerotic plaques (39). Furthermore, Bobryshev and Lord (51) observed frequent cell contacts of T cells and vascular mDC, which highly expressed HSP 70, indicating an mDC-induced T-cell activation against HSP 70.

With regard to chronic infections, the presence of Chlamydia pneumoniae was described on vascular mDCs (53). An important role of vascular mDC in the immune response to certain (auto)antigens accelerating atherogenesis. However, future studies will have to determine whether a depletion or specific suppression of DC might change the course of atherosclerosis.

**Conclusions.** Our study showed a significant decrease in circulating mDC precursors in patients with CAD, particularly those with ACS, similar to the reductions observed in autoimmune or infectious diseases. The accumulation in mDC precursors in vulnerable atherosclerotic plaques suggests that the decrease in circulating mDC precursors is caused by their recruitment into the vessel wall. Because DCs are strong T-cell stimulators, inflammation might be enhanced by their invasion into atheroma, leading to plaque progression and destabilization.

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