Monocyte heterogeneity in obesity and subclinical atherosclerosis

Kyrill S. Rogacev†‡, Christof Ulrich†‡, Lutz Blömer, Florian Hornof, Katrin Oster, Maren Ziegelin, Bodo Cremers, Yvonne Grenner, Jürgen Geisel, Axel Schlitt, Hans Köhler, Danilo Fliser, Matthias Girndt, and Gunnar H. Heine*

1Department of Internal Medicine IV, Saarland University Hospital, Homburg 66421, Germany; 2Department of Internal Medicine III, Saarland University Hospital, Homburg, Germany; 3Department of Internal Medicine II, Saarland University Hospital, Homburg, Germany; 4Central Clinical Chemistry Laboratory, Saarland University Hospital, Homburg, Germany; and 5Department of Internal Medicine III, Martin-Luther-University, Halle/Saale, Germany

Received 22 September 2008; revised 14 June 2009; accepted 15 July 2009; online publish-ahead-of-print 17 August 2009

Aims
Monocytes and monocyte-derived macrophages have been recognised as the cellular hallmark of atherosclerosis decades ago. Recently, they have also been shown to play a pivotal role in obesity. Monocytes display immunophenotypic heterogeneity with functionally distinct subpopulations. We initiated the I LIKE HOMe study to examine monocyte heterogeneity in obesity and subclinical atherosclerosis.

Methods and results
We assessed carotid intima media thickness (IMT), body mass index (BMI), and other cardiovascular risk factors in 622 healthy volunteers. Using flow-cytometry, we differentiated monocytes into CD14⁺CD16⁻ and CD16⁺ cells, which we further subdivided into CD14⁺CD16⁺ and CD14(⁻)CD16⁺ cells. Body mass index was significantly correlated with carotid IMT. High CD16⁺ monocyte counts were significantly associated with both higher BMI and increased carotid IMT. Adjustment for CD16⁺ monocyte counts weakened the correlation between BMI and carotid IMT, suggesting that the increase in CD16⁺ monocyte numbers in obesity may partly explain the association between obesity and IMT.

Conclusion
Our results reveal a significant univariate association between CD16⁺ monocytes and both obesity and subclinical atherosclerosis in low-risk individuals. They are in line with recent observations that CD16⁺ monocytes show high endothelial affinity and a potent capacity to invade vascular lesions and to transform into pro-inflammatory cytokine producing macrophages.

Keywords
Immunology • Monocytes • Subclinical atherosclerosis • Obesity

Introduction
Obesity is a worldwide growing epidemic with enormous medical and socioeconomic impact. Decades ago obesity has been described as a strong cardiovascular risk factor in observational studies. However, scientific focus was laid only recently on obesity research, and the underlying mechanisms connecting obesity and atherosclerosis are still poorly understood.

In contrast, the pathogenesis of atherosclerosis has been studied extensively and fundamental knowledge about the involved processes has been gained. In atherosclerosis, monocytes and monocyte-derived macrophages are the cellular hallmark. After initial injury, endothelial cells secrete proinflammatory molecules which attract circulating monocytes to the nascent lesion. Within the lesion, these monocytes differentiate into macrophages, which take up modified lipoproteins and produce cytokines and chemokines that attract and activate smooth muscle cells, additional monocytes, and T-cells.

Heterogeneity among human monocytes was first described two decades ago. According to their surface expression pattern of the LPS receptor CD14 and the Fcγ receptor CD16, three human monocyte subpopulations can be defined: CD14⁺CD16⁻ cells, CD14⁺CD16⁺ cells, and CD14(⁻)CD16⁺ cells. The latter two subpopulations are summarized as CD16⁺ monocytes, which...
have traditionally been considered to represent proinflammatory monocytes, comprising 10–20% of all circulating monocytes.6,7

Very recently, animal studies demonstrated a specific contribution of certain monocyte subsets to atherosclerosis.8,9 Furthermore, we found that patients with coronary artery disease have increased numbers of CD16+ monocytes compared with healthy controls.10

Interestingly, in 2003, an obesity-associated macrophage accumulation in adipose tissue has been described in mice.11 So far, in humans, an association between monocyte heterogeneity and obesity has not been evaluated in a large epidemiological study.

In the prospective I LIKE HOMe study (Inflammation, Lipoprotein Metabolism and Kidney Damage in early atherogenesis—The Homburg Evaluation), we hypothesized that CD16+ monocytes are related to both obesity and atherosclerosis.

**Methods**

**Subjects**

The I LIKE HOMe study is a cohort study which recruited 622 health-care workers employed at the Saarland University Hospital in Homburg, Germany. Healthcare workers aged 25–60 years who were scheduled to undergo routine medical checkups were invited to an ultrasonographic examination of the carotid arteries. Participants were excluded, if they had prevalent cardiovascular disease, diabetes mellitus, active tumour disease, inflammatory/autoimmune disease requiring systemic immunosuppressive treatment, or chronic kidney disease stage 4–5 (corresponding to estimated glomerular filtration rate < 30 mL/min/1.73 m²). Prevalent cardiovascular disease was defined as a history of myocardial infarction, coronary artery angioplasty/stenting, major stroke, carotid endarterectomy/stenting, non-traumatic lower extremity amputation, or lower limb artery bypass surgery/angioplasty/stenting.

Written informed consent was obtained from all study participants. The study protocol was approved by the Ethics Committee of Saarland University.

A standardized questionnaire was used to record a history of smoking, diabetes, current drug intake, cardiovascular comorbidity, and a family history of premature onset of cardiovascular disease (defined as myocardial infarction or stroke before the age of 60 years in first-degree relatives). Anthropomorphic measurements and resting blood pressure were recorded. Body mass index (BMI) was calculated as individual’s body weight divided by the square of their height, and categorized as underweight (BMI < 20 kg/m²), normal weight (BMI 20.0–24.9 kg/m²), overweight (BMI 25.0–29.9 kg/m²), obesity class I (BMI 30.0–34.9 kg/m²), and obesity Classes II and III (BMI ≥ 35.0 kg/m²). Owing to the low number of patients with extreme obesity, obesity Classes II and III were summarized as a single category.

Systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate were measured after 5 min of rest. Mean blood pressure was calculated as DBP + [(SBP – DBP)/3], and pulse pressure was calculated as SBP – DBP.

Participants were categorized as active smokers, if they were current smokers or had stopped smoking less than 1 month before entry into the study. All participants who had stopped smoking ≥1 month before study entry were categorized as former smokers. Individuals with self-reported diabetes mellitus, with a non-fasting blood sugar level of >11.10 mmol/L, with a fasting blood sugar level of >6.99 mmol/L, or with current use of hypoglycaemic medication were categorized as diabetic and were excluded from the study.

Categories of risk for coronary heart disease (CHD) were defined by Framingham risk scoring. Ten years risk for myocardial infarction and coronary death were determined using electronic calculators, which are available on the ATP III page of the National Heart, Lung, and Blood Institute Website (www.nhlbi.nih.gov/guidelines/cholesterol). Subjects were arbitrarily stratified by their CHD risk into four categories (10 years risk <1%, 1–5%, 6–10%, and >10%).

**Biochemical analysis**

Blood samples were taken from all subjects under standardized conditions. Plasma glucose, creatinine, total cholesterol, and high-density cholesterol (HDL-C) were obtained using standard techniques. Definition and classification of chronic renal disease followed the KDQI guidelines,12 and glomerular filtration rate was calculated using the MDRD study equation (4).12

Leucocyte and monocyte counts were measured with automated cell counters by standard techniques. Monocyte subpopulations were analysed via flow cytometry in a whole blood assay using 100 µL of heparin anti-coagulated blood, as described before.13 In brief, cells were stained by monoclonal antibodies (as listed below) and measured by flow cytometry (FACSCalibur; BD Biosciences, Heidelberg, Germany) using the Cell Quest software.

Monocytes were gated in a SSC/CD86+ dotplot, identifying monocytes as CD86+ cells with monocyte scatter properties. Subsets of CD14++CD16−, CD14++CD16+ and CD14++CD16+ monocytes were defined according to the surface expression pattern of the LPS receptor CD14 and the Fcy receptor CD16 (compare figure 1 for a representative example). The following antibodies were used: CD86 [HAS.287, PE (phycoerythrin)-conjugated, Beckman-Coulter, Krefeld, Germany], CD16 [3G8, APC (allophycocyanin)-conjugated, Beckman-Coulter, Krefeld, Germany]

**Figure 1** Monocyte subsets: CD86+ cells with monocyte scatter properties were gated and monocyte subpopulations were defined according to their surface expression pattern of the LPS receptor CD14 and the Fcγ receptor CD16 (representative example).
CD16 tested for a trend of IMT measurements across increasing quintiles of serum total cholesterol, smoking, mean blood pressure) and CD16. Subjects were stratified by quintiles of CD16 and partitioning the between-subjects sums of squares into trend components. Finally, sub-one-way analysis of variances (ANOVA), partitioning the increasing predefined categories of risk for CHD, respectively, by CD16 relationship between IMT and BMI while controlling for the effect of partial correlation coefficients were calculated to describe the relation coefficients were calculated by Spearman test. In addition, categorical variables are presented as percentage of participants. Correlation coefficients were calculated by Spearman test. In addition, partial correlation coefficients were calculated to describe the relationship between IMT and BMI while controlling for the effect of CD16 monocyte counts.

We tested for a trend of IMT measurements and monocyte (subset) counts across (i) increasing predefined categories of BMI and (ii) increasing predefined categories of risk for CHD, respectively, by one-way analysis of variances (ANOVA), partitioning the between-groups sums of squares into trend components. Finally, subjects were stratified by quintiles of CD16 monocyte counts, and we tested for a trend of IMT measurements across increasing quintiles of CD16 monocyte counts.

Subsequently, a multivariate linear regression analysis was calculated, which included traditional cardiovascular risk factors (BMI, age, gender, serum total cholesterol, smoking, mean blood pressure) and CD16 monocytes as independent variables, and IMT measurements as dependent variable.

Carotid ultrasound
The intima media thickness (IMT) of the common carotid artery was measured from high-resolution, two-dimensional ultrasound images obtained by a linear-array 7.5 MHz transducer (Sonoline Siena, Siemens, Erlangen, Germany). With the subject in a supine position and the head slightly extended and turned to the opposite direction, longitudinal B-mode images of the distal common carotid artery and the carotid bulb were acquired and digitally stored for offline reading.

The offline reading process was performed by a single-blinded investigator. Intima media thickness was defined as the distance between the leading edges of the lumen interface and the media- adventitia interface of the far wall. Three representative IMT measurements were performed in the far wall of both common carotid arteries at predefined positions (1.0, 2.0, and 3.0 cm proximal to the bifurcation), and these six IMT readings were averaged to give the mean common carotid IMT. Intima media thickness was not measured at the site of a carotid plaque.

Impact of general and abdominal adiposity on monocyte heterogeneity
In the I LIKE HOME trial, the distribution of body fat was not recorded. In order to study the association of monocyte subpopulations to abdominal and gluteofemoral obesity, assessed as waist circumference and hip circumference, respectively, we recruited a second study cohort of 115 subjects who were admitted to the Department of Internal Medicine III of the Saarland University Hospital for diagnostic coronary angiography between April 2007 and March 2008. Coronary artery disease, defined as ≥50% diameter stenosis in a major coronary artery, was ruled out in all 115 subjects.

Before coronary angiography, a blood sample was drawn for characterization of monocyte subpopulations, as described above, and measurements of waist circumference (at the midpoint between the lowest rib and the iliac crest) and hip circumference (at the trochanter major) were performed. Waist–hip ratio was defined as the ratio of waist girth to the circumference of the hips.

Statistics
Data management and statistical analysis were performed with SPSS 13.0. Unless indicated otherwise, continuous data are expressed as mean ± standard deviation and compared by Mann–Whitney test. Categorical variables are presented as percentage of participants. Correlation coefficients were calculated by Spearman test. In addition, partial correlation coefficients were calculated to describe the relationship between IMT and BMI while controlling for the effect of CD16 monocyte counts.

We tested for a trend of IMT measurements and monocyte (subset) counts across (i) increasing predefined categories of BMI and (ii) increasing predefined categories of risk for CHD, respectively, by one-way analysis of variances (ANOVA), partitioning the between-groups sums of squares into trend components. Finally, subjects were stratified by quintiles of CD16 monocyte counts, and we tested for a trend of IMT measurements across increasing quintiles of CD16 monocyte counts.

Subsequently, a multivariate linear regression analysis was calculated, which included traditional cardiovascular risk factors (BMI, age, gender, serum total cholesterol, smoking, mean blood pressure) and CD16 monocytes as independent variables, and IMT measurements as dependent variable.

Results
Baseline characteristics
Among the 622 healthcare workers recruited, 569 individuals were included in the present analysis. In 28 individuals, monocyte subset determination failed due to technical reasons, three individuals had no ultrasonographic examination of the carotid arteries, and the remaining 22 individuals met one or more exclusion criteria, as they had prevalent cardiovascular disease, systemic immunosuppressive treatment, diabetes mellitus, chronic kidney disease stage 4–5, or were aged more than 60 years.

The baseline characteristics of these 569 subjects are shown in Table 1. Three hundred and sixty-two participants were female (63.6%), 101 participants had a family history of premature-onset cardiovascular disease (17.8%), and 175 subjects were current smokers (30.8%).

Body mass index and subclinical atherosclerosis
Mean BMI was 25 ± 4 kg/m². Thirty-nine individuals were categorized as underweight (BMI < 20 kg/m²), 282 individuals had normal weight (BMI 20.0–24.9 kg/m²), 183 individuals were overweight (BMI 25.0–29.9 kg/m²), and 65 individuals were obese [46 participants with obesity Class I (BMI 30.0–34.9 kg/m²), 19 participants with obesity Classes II and III (BMI ≥ 35.0 kg/m²)].

Mean IMT was 0.43 ± 0.08 mm (range 0.28–0.93 mm). Body mass index was significantly correlated with carotid IMT measurements (r = 0.345, P < 0.001). Subjects with underweight and

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Baseline characteristics of I LIKE HOMe participants (n = 569)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
</tr>
<tr>
<td>Age (years)</td>
<td>42</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>73</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>131</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>85</td>
</tr>
<tr>
<td>Pulse pressure (mmHg)</td>
<td>46</td>
</tr>
<tr>
<td>Mean blood pressure (mmHg)</td>
<td>100</td>
</tr>
<tr>
<td>Physical activity (units/week)</td>
<td>1.5</td>
</tr>
<tr>
<td>Alcohol intake (drinks/week)</td>
<td>2.9</td>
</tr>
<tr>
<td>Serum creatinine (µmol/L)</td>
<td>79.6</td>
</tr>
<tr>
<td>Leucocytes/µL</td>
<td>6760</td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>5.13</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>1.74</td>
</tr>
</tbody>
</table>

*aActivity strenuous enough to build up a sweat, and lasting ≥ 30 min.
*bOne drink = 350 mL of beer, 120 mL of wine, or 45 mL of spirits.
normal weight had significantly lower IMT compared with subjects with overweight and obesity (ANOVA with post hoc Scheffé test; Figure 2).

In addition, IMT measurements were significantly correlated with traditional cardiovascular risk factors [age: $r = 0.500; P < 0.001$; mean blood pressure: $r = 0.394; P < 0.001$; total cholesterol: $r = 0.187; P < 0.001$; HDL-C: $r = (-0.195); P < 0.001$]; IMT measurements were higher in male participants than in females ($0.45 \pm 0.09$ mm vs. $0.42 \pm 0.07$ mm; $P < 0.001$), and in former or current smokers ($0.44 \pm 0.08$ mm) than in participants who had never smoked ($0.42 \pm 0.07$ mm; $P = 0.011$). As a consequence, we found a significant association between subclinical atherosclerosis and Framingham risk score-based categories of risk for CHD (Figure 3).

**Figure 2** Intima media thickness by categories of body mass index (indicated are the mean and the standard error of the mean).

**Figure 3** Intima media thickness by categories of risk for coronary heart disease (indicated are the mean and the standard error of the mean).

**Body mass index and leucocyte subpopulations**

The I LIKE HOMe participants had a mean of $6760 \pm 1884$ leucocytes/μL and $486 \pm 168$ monocytes/μL ($7.3 \pm 1.9\%$ of all leucocytes), of which $405 \pm 147$ cells ($83.1 \pm 6.4\%$ of all monocytes) were CD16$^+$ monocytes, and $80 \pm 42$ cells were CD16$^-$ monocytes ($16.9 \pm 6.4\%$ of all monocytes). Among CD16$^+$ monocytes, $20 \pm 13$ cells ($4.1 \pm 1.7\%$ of all monocytes) were CD14$^+$CD16$^+$ cells, and $60 \pm 33$ cells ($12.8 \pm 5.6\%$ of all monocytes) were CD14$^{++}$CD16$^+$ cells.

Body mass index was weakly correlated with total leucocyte counts, but not with total monocyte count. Taking monocyte heterogeneity into account, a positive correlation was found between BMI and CD16$^+$ monocytes, but not CD16$^-$ monocytes. When further subdividing CD16$^+$ monocytes into CD14$^+$CD16$^+$ and CD14$^{++}$CD16$^+$ monocytes, only the latter subpopulation was significantly associated with BMI (Table 2).

Stratification of study participants by BMI into five predefined categories revealed a progressive increase in CD16$^+$ monocyte counts (Figure 4), but not in total monocyte counts or in CD16$^-$ monocyte counts (data not shown): Individuals with obesity Class II/III had almost a doubling in CD16$^+$ monocytes compared with underweight individuals.

In addition, CD14$^+$CD16$^+$ monocyte counts showed a weak, albeit significant correlation with the traditional cardiovascular risk factors increasing age and higher blood pressure, whereas total monocytes, CD16$^+$ monocytes, and CD14$^{++}$CD16$^+$ monocytes did not (Table 2).

When calculating categories of risk for CHD, higher Framingham risk scores were associated with higher CD16$^+$ monocyte counts (Figure 5).

**CD16$^+$ monocytes and subclinical atherosclerosis**

When stratifying subjects by quintiles of CD16$^+$ monocyte counts, IMT values significantly rose with higher CD16$^+$ monocyte counts (Figure 6). In contrast, neither total monocyte counts nor monocyte counts of CD16$^-$ monocytes were associated with IMT measurements (data not shown).

We recalculated the association between BMI and IMT after controlling for CD16$^+$ cell counts in a partial correlation procedure. The resulting correlation coefficient was $r = 0.282$, which was lower than the univariate correlation coefficient of $r = 0.345$ (both $P < 0.0001$), suggesting that the increase in CD16$^+$ monocyte numbers in obesity may partly explain the association between obesity and subclinical atherosclerosis.

Finally, we calculated a multivariate linear regression analysis, which included traditional cardiovascular risk factors, BMI and CD16$^+$ monocyte counts as independent variables and IMT as dependent variable (Table 3). Body mass index was independently associated with IMT measurements after adjustment for age and gender (Model 1), as well as after additional adjustment for CD16$^+$ monocyte counts (Model 2). When further adjusting for total cholesterol levels, arterial blood pressure, and current smoking (Model 3), the association between BMI and IMT
measurements is attenuated, but remains of marginal significance ($P = 0.077$).

When including age and CD16$^+$ monocyte counts, rather than BMI, into Model 1, CD16$^+$ monocytes are independently associated with IMT measurements ($\beta = 0.076; P = 0.038$). After inclusion of BMI into this model, CD16$^+$ monocyte counts no longer independently predict IMT measurements (data not shown). Similarly, CD16$^+$ monocyte counts are not independently associated with IMT measurements when adjusting for Framingham risk score (data not shown).

### Impact of general and abdominal adiposity on monocyte heterogeneity

The association between monocyte subset counts and the distribution of body fat was assessed in a second cohort of 115 patients with angiographic exclusion of coronary artery disease. Mean age of these 115 subjects was 63.4 ± 11.4 years, and mean BMI was 28.0 ± 4.8 kg/m$^2$. In agreement with the I LIKE HOMe study results, BMI was significantly correlated with CD16$^+$ ($r = 0.306,$ $P = 0.002$).

$\begin{array}{ccc}
\text{Table 2 Univariate correlates of total leucocyte counts and counts of leucocyte and monocyte subsets with traditional and non-traditional cardiovascular risk factors} \\
\hline
& \text{Total leucocytes} & \text{Total monocytes} & \text{CD16}^- \text{ monocytes} & \text{CD16}^+ \text{ monocytes} & \text{CD14}^{++} \text{CD16}^+ \text{ monocytes} & \text{CD14}^{+/-} \text{CD16}^+ \text{ monocytes} \\
\hline
r & P-value & r & P-value & r & P-value & r & P-value & r & P-value & r & P-value \\
\hline
\text{Body mass index} & 0.130 & 0.002 & 0.054 & 0.195 & 0.012 & 0.783 & 0.216 & <0.001 & 0.057 & 0.175 & 0.249 & <0.001 \\
\text{Mean BP} & 0.106 & 0.012 & 0.068 & 0.106 & 0.033 & 0.439 & 0.175 & <0.001 & 0.057 & 0.172 & 0.196 & <0.001 \\
\text{Age} & 0.111 & 0.008 & 0.040 & 0.343 & 0.018 & 0.676 & 0.122 & 0.004 & 0.004 & 0.917 & 0.149 & <0.001 \\
\text{Total cholesterol} & 0.024 & 0.563 & -0.022 & 0.606 & -0.037 & 0.383 & 0.057 & 0.171 & -0.025 & 0.545 & 0.084 & 0.046 \\
\text{HDL-C} & -0.087 & 0.037 & -0.070 & 0.093 & -0.054 & 0.198 & -0.098 & 0.019 & -0.067 & 0.109 & -0.095 & 0.023 \\
\hline
\end{array}$

$r$, Correlation coefficients, calculated by Spearman test; $P$-value, level of significance. Significant correlations after adjustment for multiple testing are in bold face.
Table 3  Multiple linear regression analysis with carotid intima media thickness as dependent variable

<table>
<thead>
<tr>
<th>Dependent variable: IMT (mm)</th>
<th>Model 1</th>
<th></th>
<th>Model 2</th>
<th></th>
<th>Model 3</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>β</td>
<td>P-value</td>
<td>β</td>
<td>P-value</td>
<td>β</td>
<td>P-value</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.130</td>
<td>0.001</td>
<td>0.127</td>
<td>0.001</td>
<td>0.071</td>
<td>0.077</td>
</tr>
<tr>
<td>Age (years)</td>
<td>0.469</td>
<td>&lt;0.001</td>
<td>0.468</td>
<td>&lt;0.001</td>
<td>0.415</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>−0.191</td>
<td>&lt;0.001</td>
<td>−0.188</td>
<td>&lt;0.001</td>
<td>−0.153</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>CD16⁺ monocyte counts (cells/μL)</td>
<td>0.022</td>
<td>0.543</td>
<td>0.015</td>
<td>0.678</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td></td>
<td></td>
<td>0.025</td>
<td>0.504</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean arterial blood pressure (mmHg)</td>
<td></td>
<td></td>
<td>0.171</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current smoking (yes)</td>
<td></td>
<td></td>
<td>0.030</td>
<td>0.395</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \beta \), standardized regression coefficient; \( P \)-value, level of significance.

\( P = 0.001 \) and CD14(+)CD16⁺ monocyte counts \( r = 0.319, P = 0.001 \). Hip circumference \( r = 0.256, P = 0.006 \) and waist circumference \( r = 0.263; P = 0.005 \) correlated to a similar degree with CD16⁺ monocyte counts. As a result, the waist–hip ratio was not significantly associated with CD16⁺ monocyte counts \( r = 0.102; P = 0.279 \). Total and CD14⁺⁺CD16⁺ monocyte counts were neither correlated with BMI nor with hip and/or waist circumference (data not shown).

**Discussion**

Obesity and atherosclerosis have both been characterized as chronic inflammatory diseases.

As established decades ago, monocytes and macrophages are the driving force in atherosclerosis—from earliest morphological atherosclerotic changes until final plaque rupture with potentially devastating outcome.²⁻⁴

In analogy, monocyte-derived macrophages have also been shown to accumulate in adipose tissue of obese mice, where they contribute to a dysfunctional state with subsequent overexpression of proinflammatory cytokines and peripheral insulin resistance.⁵

Of note, Passlick et al.⁵ demonstrated in 1989 that morphologically and functionally distinct monocyte subsets exist: the majority of circulating monocytes are characterized by the surface expression of the LPS receptor CD14, whereas not expressing the FcγRII receptor CD16 (CD16⁻ monocytes). A minority of monocytes co-expresses CD14 and CD16 (CD16⁺ monocytes). Subsequently, CD16⁺ monocytes were shown to be potent producers of pro-inflammatory cytokines, and an expansion of this subset was noted in multiple inflammatory disorders, e.g. sepsis, HIV infection, and tuberculosis, as recently reviewed.⁶

Surprisingly, the role of CD16⁺ monocytes in the inflammatory disease atherosclerosis was ignored until recently. In 2003, we reported a shift in monocyte subsets towards CD16⁺ monocytes in patients with CHD compared with healthy controls.⁷ Subsequently, the evaluation of the role of CD16⁺ monocytes in early atherogenesis has been suggested as a research priority.⁸

Not long ago, monocyte heterogeneity, monocyte heterogeneity was established in a murine model, enabling researchers to study monocyte subsets in models of atherosclerosis. In two recent publications, a specific contribution of certain monocyte subsets to atherogenesis was confirmed.⁹⁻¹⁰

Lately, Auffray et al.¹⁹ demonstrated that the murine counterparts of CD16⁺ monocytes (Ly6C⁻ monocytes) exhibit a ‘patrolling behaviour’, which is characterized by firm attachment to endothelial cells, crawling along the endothelium, and rapid extravasation following inflammatory stimuli. Once extravasated, Ly6C⁻ monocytes start to secret proinflammatory cytokines and develop into macrophages. The authors hypothesized a selective role of this monocyte subset in atherosclerosis and considered these cells to be a potential therapeutic target.¹⁹

The I LIKE HOMe study reveals a significant association between counts of CD16⁺ monocytes—but not of total monocytes or CD16⁻ monocytes—and both obesity as well as subclinical atherosclerosis in a large cohort of healthy individuals. The association of CD16⁺ monocyte counts with obesity is in line with a study in patients with morbid obesity.²⁰ When compared with healthy controls, 27 morbidly obese patients had a shift towards CD16⁺ monocytes. Bariatric surgery with subsequent weight loss led to the lowering of pre-interventional elevated CD16⁺ monocyte counts.

The findings of our study might explain the seemingly puzzling results of previous large cohort studies which could not unanimously find a significant correlation between total monocyte counts and IMT despite the pivotal role of monocytes in atherogenesis.²¹,²²

Similar to the distinction of different monocyte subpopulations, heterogeneity among macrophages has gained substantial interest in the last years. As outlined before, adipose tissue macrophages contribute to the metabolic disturbances due to obesity. The exact functional and phenotypic characterization of adipose tissue macrophage subpopulations is under current, controversial investigation.²³ For example, Bourlier et al.²⁴ identified CD16⁺ macrophages within stroma-vascular fraction cells (SVFC) in adipose tissue, whereas Zeyda et al.²⁵ did not find CD16⁺ cells within these SVFC.

It is strongly debated how monocyte heterogeneity relates to the different macrophage activation states.²⁶ Specifically, it is yet unclear whether a transition from a distinct monocyte...
subpopulation to a specific macrophage type exists. Even if this subset-specific transition existed, it remains to be elucidated which monocyte subpopulation would give rise to which macrophage type.23

Therefore, it is currently very difficult to put our results about peripheral blood monocyte heterogeneity in obesity and subclinical atherosclerosis into context with studies on adipose tissue macrophages. Thus, our data do neither confirm nor refute the recent findings by Bourlier et al.24 who found a positive correlation between CD45+CD14+CD206+CD16+ cells and BMI. In 2003, it was appreciated that CD16+ monocytes can be subdivided into CD14+CD16+ and CD14+CD16+ cells,26 even though the biological significance of this distinction is still unclear. Since in the present study, the association between monocyte subpopulations with obesity as well as with subclinical atherosclerosis was uniquely conferred by CD14+CD16+ cells—similar to our earlier findings in renal transplant recipients on a steroid-free immunosuppressive regimen27—differences between both subsets of CD16+ monocytes need to be more thoroughly investigated in the future.

Analysing our epidemiological data in the light of previously published experimental findings,8,9 we propose the following hypothesis as a possible explanation for our results: obesity might exert its atherogenic potential partly via a shift from CD14+ monocytes to CD16+ monocytes, which in turn might accelerate atherogenesis by their previously described pro-atherosclerotic virtues, which include the preferential attachment to activated endothelial cells,26 their ability to migrate into the vessel wall, to differentiate into macrophages,19 and to secrete proinflammatory cytokines.16

Future studies are needed to test this hypothesis. These studies should especially aim to delineate mechanisms by which adipose tissue affects monocyte differentiation, and whether therapeutic interventions such as weight reduction by life-style modification or pharmacotherapy exert their beneficial physiological effects partly by normalizing monocyte subset distribution.

Given its nature as a cohort study, the I LIKE HOME study can merely show associations, but not prove causal relations. In order to examine the prognostic impact of increased CD16+ monocyte counts on future cardiovascular events, we are currently initiating a prospective study in patients with manifest CHD.

In the present study, we deliberately decided to recruit a cardiovascular low-risk population in order to study early stages of atherosclerosis. This imposes a further limitation on our study, as measurements of IMT were virtually within normal ranges, and thus carotid ultrasound may represent a crude tool to assess early atherosclerosis. Therefore, correlation coefficients between IMT and monocyte subsets were expected to be moderate. Nevertheless, the predefined level of significance was achieved in univariate analysis. As in our study, other cohort studies that recruited comparable low-risk individuals found only weak associations between IMT and other biomarkers for atherosclerosis.28

Not surprisingly, the association of CD16+ monocyte counts and BMI with subclinical atherosclerosis failed to achieve statistical significance in a linear multiple regression analysis after adjustment for traditional cardiovascular risk factors. This does not preclude a causal link between inflammation, obesity, and subclinical atherosclerosis, as some of these traditional risk factors—notably serum cholesterol levels and arterial blood pressure—are affected by obesity. Thus they should be considered as mediators of the presumed pro-atherosclerotic potential of obesity and inflammation, rather than as confounders.

In summary, we show for the first time a subset-specific relation between CD16+ monocytes and both subclinical atherosclerosis as well as obesity in a large cohort of healthy volunteers. We thus provide epidemiological data for a role of CD16+ monocytes in the relation between obesity and atherosclerosis.

Acknowledgement

The skilful technical assistance of Martina Wagner is greatly appreciated.

Funding

The study was partly funded by intramural research grants (“HOMFOR 2004/2005”).

Conflict of interest: none declared.

References