Distinct scavenger receptor expression and function in the human CD14+/CD16+ monocyte subset

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Distinct scavenger receptor expression and function in the human CD14+/CD16+ monocyte subset. Am. J. Physiol. 276 (Heart Circ. Physiol. 45): H1144–H1149, 1999.—The CD14+/CD16+ subset of human blood monocytes, which expresses low levels of the lipopolysaccharide receptor CD14 and high levels of the Fc receptor CD16 and exhibits features of mature tissue macrophages, is expanded in certain inflammatory conditions and may be relevant in atherosclerosis. Scavenger receptors (ScR) are important for lipid accumulation into macrophage-derived foam cells in atherogenesis and for the clearance of pathogens. Hence, we compared the function and expression of ScR in CD33low CD16+ and CD33high CD14+/CD16+ monocyte subsets. Double immunofluorescence analysis of isolated monocytes revealed that the CD33low subset showed lower specific, ScR-mediated binding of DiI-labeled modified low-density lipoproteins (LDL) than CD33high cells. Differences in modified LDL binding between subsets were accompanied by changes in mRNA expression. RT-PCR in sorted cells indicated lower ScR class A type I/II (ScR-AI/II) mRNA levels in CD14+/CD16+ than in CD14+ cells, whereas CD36 transcripts were unaltered. This was paralleled by findings in mostly CD16+ monocyte-derived macrophages showing a marked reduction in ScR-mediated binding of acetylated LDL, but not in the binding of oxidized LDL, and lower expression of ScR-AI/II mRNA, but not CD36 transcripts, after exposure to tumor necrosis factor-α for 48 h in vitro. Thus the subset of CD14+/CD16+ monocytes shows distinct ScR function and expression, possibly reflecting a preactivation by cytokines with a predilection for specific inflammatory or vascular conditions, e.g., atherogenesis.

Variations in the expression of cell surface molecules reflect preactivation by cytokines with a predilection for specific inflammatory or vascular conditions, e.g., atherogenesis; scavenger receptors; monocytes; oxidized low-density lipoproteins

Tissue macrophages are characterized by a broad heterogeneity in phenotype and function, whereas less is known about distinct human blood monocyte subpopulations. Recently, a subset of blood monocytes (CD14+/CD16+) has been identified that expresses low levels of CD14, the receptor for lipopolysaccharide (LPS), and high levels of CD16, the Fc receptor II (19, 39, 41). On the basis of expression of the two molecules, these cells resemble alveolar but not peritoneal macrophages, which maintain high levels of CD14 and are negative for CD16. Indeed, a detailed analysis of CD14+/CD16+ monocytes revealed markedly higher class II expression and lower expression of the CD11b receptor and the panmyeloid differentiation marker CD33, similar to alveolar or monocyte-derived macrophages generated in vitro (40), confirming that CD14+/CD16+ cells have acquired features of more differentiated monocytes or even mature tissue macrophages. The CD14+/CD16+ monocyte subset was found to be expanded in patients with human immunodeficiency virus-1 infection and in sepsis, in which expansion is preceded by production of the monocyte-activating cytokines tumor necrosis factor (TNF)-α, monocyte colony-stimulating factor (MCSF), or interleukin (IL)-6 (3, 8, 16). On the other hand, CD14+/CD16- or CD33low monocyte subsets express high levels of proinflammatory TNF-α but no detectable levels of anti-inflammatory IL-10 in response to LPS stimulation (9). It has also been suggested that systemic abnormalities in mononuclear phagocyte subpopulations may serve as cellular markers in hypercholesterolemia, an established risk factor for atherosclerosis (22). Monocyte infiltration is crucially involved in chronic inflammatory reactions and in the pathogenesis of atherosclerosis, in which lipid accumulation in monocyte-derived macrophages via scavenger receptors (ScR) results in the development of foam cells and progression of the lesion (2, 21, 38). ScR are a functionally defined group of proteins that mediate binding and uptake of modified low-density lipoprotein (LDL) and subsequent cholesterol esterification and that may be involved in the clearance of pathogens (e.g., LPS) in endotoxic shock (10, 13, 25, 38). LPS and inflammatory cytokines have been shown to regulate ScR expression in the monocyte lineage and may be involved in the expansion of CD14+/CD16+ cells (12, 30). Here we report that the CD33low CD14+/CD16+ monocyte subset shows impaired ScR-dependent binding of modified LDL and reduced expression of ScR class A type I/II (ScR-AI/II) mRNA. This was paralleled by effects of TNF-α in monocyte-derived macrophages and may reflect preactivation by cytokines occurring, for example, under certain inflammatory conditions associated with vascular disease.

MATERIALS AND METHODS

Cell isolation and preparation of modified lipoproteins. Peripheral blood mononuclear cells were isolated from leukocyte-rich plasma of apparently healthy donors by Ficoll-Hypaque density gradient and plastic adherence or by hypotonic lysis and centrifugation (Nycomed, Oslo, Norway) and were separated from platelets by four washes at 300 g, as described previously (9, 31). Oxidized (oxLDL) and acetylated LDL (acLDL) were pre-
pared from LDL isolated from normolipidemic donors by standard protocols, as described previously (7, 24), and were labeled with 1,1'-dioctadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI; Molecular Probes) according to the manufacturer's instructions at 37°C in the presence of lipoprotein-deficient serum (30 mg protein/ml) and ascorbic acid (100 µM), as previously described in detail (28). Labeled modified LDL was resolated by ultracentrifugation and extensively dialyzed. All reagents were from Sigma Chemical or Boehringer Mannheim unless otherwise stated.

Immunofluorescence and cell sorting. Flow cytometry on isolated monocytes was essentially performed as described previously (9, 31, 34, 40). To block unspecific binding via Fc receptors, monocytes were preincubated for 20 min with 5% human serum. For double immunofluorescence monitoring of modified LDL uptake and surface markers, cells were incubated for 1 h with DiI-labeled oxLDL or acLDL (10 µg/ml) in the absence or presence of unlabeled surplus (250 µg/ml) or fucoidan (100 µg/ml), respectively, and stained with the fluorescein isothiocyanate (FITC)-conjugated CD33 monoclonal antibody (MAb) My9 or isotype control (Coulter) for 30 min on ice. After appropriate compensation, cells were analyzed by flow cytometry in a FACSscan (Becton Dickinson) with light scatter gates for monocytes. After the binding in the presence of surplus or fucoidan was subtracted, specific binding of oxLDL and acLDL was expressed as specific mean fluorescence intensity in channels. Preparative cell sorting of monocytes stained with FITC-conjugated CD14 MAb My4 (Coulter) and phycoerythrin-conjugated CD16 MAb Leu11c (Becton Dickinson) under saturating conditions on ice was performed with an EPICS V 753 flow cytometer (Coulter) with 488-nm excitation, as described previously (9, 40). Purities were determined to be > 95% on reanalysis.

Reverse transcription-polymerase chain reaction. Sorted cells were immediately lysed with 200 µl of RNAzol B (WAK-Chemie). After the addition of carrier tRNA (5 µg), total RNA was isolated from 2 × 10⁴ cells by phenol-chloroform-isooamyl alcohol extraction and mRNA was reverse transcribed. cDNA was amplified with Taq DNA polymerase and primers by 36 cycles in a Thermocycler 480 (Perkin-Elmer Cetus) set to 95°C melting (30 s), 58°C annealing (60 s), and 72°C extension (60 s). Primers were synthesized according to published sequences with minimal homology to yield products of 366 bp for ScR-AI/II (24), 370 bp for CD36 (20), or 548 bp for β-actin (32). As described in detail elsewhere (20, 32, 33, 35), PCR products were analyzed by agarose gel electrophoresis and were quantitated by ultraviolet detection at 260 nm after separation by HPLC on a DEAE ion-exchange column (Perkin-Elmer) with a solvent gradient of 300–600 mM NaCl buffered at pH 9.0. Respective peaks appeared at retention times predicted by DNA molecular weight marker XIII (Boehringer Mannheim), and areas under the peak were integrated. For normalization, ScR-AI/II and CD36 mRNA expression data were expressed as percentages of β-actin PCR products.

RESULTS

Binding of modified LDL to monocyte subsets. To study monocyte activities relevant to the scavenging and clearance of lipids and LPS, we compared the binding of DiI-labeled modified LDL with monocyte subsets. To analyze functional differences, we exploited the fact (9) that CD14⁻ monocytes express high levels of the panmyeloic marker CD33 (CD33high), whereas the CD14⁺/CD16⁺ cells exhibit low levels of CD33 expression (CD33low). Hence, we used double immuno-
oxLDL was slightly decreased in the CD33<sub>low</sub> cells, and thus mediated by ScR-AI/II (Figs. 1 and 2, respectively). Moreover, the specific binding of DiI-labeled acLDL that was inhibitable by fucoidan, which specifically blocks interactions with ScR-AI/II products in CD14<sup>+</sup> cells, was found that the expression of ScR-AI/II mRNA was significantly reduced in CD14<sup>+</sup>/CD16<sup>+</sup> cells compared with CD14<sup>+</sup>/CD16<sup>+</sup> monocytes, as analyzed by agarose gel electrophoresis (Fig. 3) and HPLC quantification (Fig. 4A, P < 0.05). Representative HPLC chromatograms show significantly higher peak areas corresponding to ScR-AI/II products in CD14<sup>+</sup>/CD16<sup>+</sup> monocytes (Fig. 4B) compared with CD14<sup>+</sup>/CD16<sup>+</sup> cells (Fig. 4C). In contrast, mRNA expression of the combined adhesion and ScR class B CD36 was found to be unaltered (Fig. 4A), indicating that it did not contribute to the differences in binding of modified LDL shown herein. In conclusion, the reduction in binding of modified LDL in CD33<sup>low</sup>/CD16<sup>+</sup> monocytes was associated with concomitant changes in ScR-A mRNA expression, likely representing the underlying regulatory mechanism.

Association of differences in binding of modified LDL with changes in mRNA expression. To assess whether differential binding of modified LDL in the monocyte subsets may be accompanied by changes in mRNA expression, we performed RT-PCR analysis and HPLC quantification using mRNA isolated from cells that were separated into the two subsets with >95% purity by fluorescence-activated cell sorting. Consistent with the lower ScR-mediated binding of modified LDL in CD33<sup>low</sup>/CD16<sup>+</sup> cells, we found that the expression of ScR-AI/II mRNA was significantly reduced in CD14<sup>+</sup>/CD16<sup>+</sup> cells compared with CD14<sup>+</sup>/CD16<sup>+</sup> monocytes, as analyzed by agarose gel electrophoresis (Fig. 3) and HPLC quantification (Fig. 4A, P < 0.05). Representative HPLC chromatograms show significantly higher peak areas corresponding to ScR-AI/II products in CD14<sup>+</sup>/CD16<sup>+</sup> monocytes (Fig. 4B) compared with CD14<sup>+</sup>/CD16<sup>+</sup> cells (Fig. 4C). In contrast, mRNA expression of the combined adhesion and ScR class B CD36 was found to be unaltered (Fig. 4A), indicating that it did not contribute to the differences in binding of modified LDL shown herein. In conclusion, the reduction in binding of modified LDL in CD33<sup>low</sup>/CD14<sup>+</sup>/CD16<sup>+</sup> monocytes was associated with concomitant changes in ScR-A mRNA expression, likely representing the underlying regulatory mechanism.

Exposure to TNF-α downregulates acLDL binding and ScR-AI/II expression. The CD14<sup>+</sup>/CD16<sup>+</sup> monocyte subset appears to be expanded during inflammatory conditions, such as septicemia, involving increased levels of LPS and inflammatory cytokines (3), which have been shown to regulate chemokine and ScR expression in cells of the monocyte lineage (12, 18, 30). Hence, we investigated whether exposure of in vitro generated monocyte-derived macrophages to TNF-α for 48 h was associated with changes in acLDL binding and ScR-AI/II mRNA expression similar to those found in CD14<sup>+</sup>/CD16<sup>+</sup> cells. The in vitro culture of human monocytes for 48 h resulted in a substantial increase in the percentage of CD16-positive cells (71 ± 10 vs. 13 ± 3% in freshly isolated cells) and in a slight upregulation of the fucoidan-inhibitable DiI-acLDL binding mediated by ScR-A (compare Figs. 2 and 5A), consistent with a downregulation in macrophages. Treatment with TNF-α for 48 h significantly reduced the specific binding of DiI-labeled acLDL to ScR blocked with fucoidan to levels comparable with those in CD14<sup>+</sup>/CD16<sup>+</sup> cells (Fig. 5A). In contrast, binding of oxLDL was not significantly affected by TNF-α (data not shown). In accordance with these findings, the expression of ScR-A mRNA was downregulated in monocyte-derived macrophages by treatment with TNF-α for 48 h (Fig. 5B), indicating that reduced acLDL binding may be due to a...
downregulation of ScR-A mRNA expression. For αxLDL binding, expression of CD36 mRNA was not affected by treatment with TNF-α (data not shown). Thus our data confirm findings on the regulation of ScR expression by inflammatory cytokines and suggest that the distinct ScR function and expression of the CD14+/CD16+ monocyte subset may reflect a preactivation by cytokines.

DISCUSSION

We have found that the subset of CD33low CD16+ monocytes showed a markedly reduced expression of ScR-AI/II and, consequently, impaired functions, i.e., lower binding of modified LDL, compared with the predominant population of CD33high CD14+ monocytes. The CD33low CD16+ monocyte subset showed a slightly reduced specific binding of αxLDL and substantially lower binding of acLDL that was inhibitable by fucoidan. The latter specifically reflects the function...
of ScR-AI/II, which can account for binding and uptake of oxLDL and acLDL in vitro and in vivo (10, 25). These data were confirmed by a parallel downregulation in ScR-AI/II mRNA expression, which may result in decreased surface expression and function. Evidence has accumulated showing that LPS, various cytokines, and growth factors are involved in regulating the expression of ScR, such as ScR-AI/II or CD36, in cells of the monocyte lineage. Macrophage ScR activity and mRNA expression are inhibited (due to transcriptional or posttranscriptional mechanisms) by the inflammatory cytokines interferon-γ and by TNF-α, which also mediates the downregulation by LPS (12, 14, 30). Moreover, ScR-AI/II mRNA expression and function were suppressed by granulocyte MCSF or transforming growth factor-β in monocyte-derived macrophages but were selectively enhanced by M-CSF and differentially increased during monocyte-macrophage differentiation (4, 5, 11, 17, 29). Hence, the downregulation of ScR-AI/II expression and function in CD14+/CD16- cells may reflect an exposure of circulating monocytes to LPS or inflammatory cytokines. This is an intriguing concept, because the expansion of CD14+/CD16- cells has been shown to follow an increase in inflammatory cytokines (3) and because ScR-A on activated macrophages has been implicated in LPS scavenging and clearance in endotoxic shock, which thus may precede its downregulation due to internalization (13).

We did not observe significant differences in total binding of acLDL or oxLDL or in CD36 surface and mRNA expression between CD33low CD14+ or CD16+ and CD33high CD14++ cells, respectively. In addition to ScR-AI/II and CD36, other ScR class B members, CD68 or LOX-1 (a recently identified lectin-like receptor), may mediate binding and uptake of modified LDL in monocytes (6, 23). In contrast to differences in ScR-A, these findings thus may be due to differences in expression or function of other ScR between the subsets. The thrombospondin receptor CD36, which mediates oxLDL uptake, as revealed with a blocking MAb and by genetic deficiency, is transiently upregulated during monocyte maturation, maintained or increased by M-CSF, but downregulated by LPS (15, 18, 37). Whereas ScR regulation by single cytokines or growth factors has been established, production of TNF-α, IL-6, and M-CSF preceding septic expansion of CD14+/CD16- cells may expose circulating monocytes to multiple cytokines. The emergence or activation of CD14+/CD16- cells induced by cytokine combinations in vivo may thus be accompanied by differential effects on ScR, i.e., downregulation of ScR-AI/II by TNF-α may prevail over an enhancement by M-CSF, whereas effects on CD36 may be balanced.

Our results further show that differentiation of monocytes by in vitro culture over 48 h resulted in a macrophage-like CD16- phenotype with a predominant CD16 expression and an upregulation of ScR-A function, consistent with previous findings (5, 40). In parallel with the data in CD14+/CD16- cells, which are expanded after an increase in the levels of inflammatory cytokines in vivo (3, 8), exposure of monocytes to TNF-α in vitro caused a marked reduction in fucoidan-blocked acLDL binding and ScR-A mRNA expression. Together, our results suggest that CD14+/CD16- cells represent cytokine-activated rather than predifferentiated monocytes.

Monocyte infiltration is crucially involved in atherogenesis (21, 38), and in atherosclerotic plaques, lipid accumulation in monocyte-derived macrophages via ScR results in development of foam cells and lesion progression (10, 21, 25). In addition to our findings on ScR expression and function, we have found that the expression and function of CCR2, the receptor for the CC chemokine monocyte chemotactic protein (MCP-1), is reduced in CD14+/CD16- cells (unpublished observations), which again parallels results after treatment of monocytes with LPS or inflammatory cytokines (26, 27). Because MCP-1 has been detected in macrophage-rich areas of atherosclerotic plaques (38), exposure of monocytes to inflammatory cytokines present in atherosclerotic lesions (21) may result in a phenotype resembling CD14+/CD16- cells with impaired CCR2 and ScR expression and function, providing a physiological mechanism to limit excessive MCP-1-driven migration and recruitment of monocytes into such inflammatory sites (unpublished observations) with subsequent lipid accumulation and foam cell development. On the other hand, abnormalities in monocyte subpopulations have been proposed to serve as cellular characteristics in hypercholesterolemia, a risk factor for atherosclerosis (22). Hence, an expansion of CD14+/CD16- monocytes may reflect a preactivation by inflammatory cytokines, resulting in a phenotype with specific ScR and chemokine receptor expression, and may be a novel and useful cellular indicator of acute or chronic inflammatory conditions, such as sepsis and immunodeficiency, or of the inflammatory activity in vascular disease and atherogenesis.

We thank Profs. R. Lorenz and P. C. Weber for continuous support and Drs. K. S. C. Weber and N. Hrboticky for help with monocyte and low-density lipoprotein preparation.

This work was supported by grants from the Deutsche Forschungsgemeinschaft (We 1913-2 to C. Weber) and August Lenz-Stiftung. It partially fulfills requirements for the doctoral thesis of G. Draude and P. von Hundelshausen.

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Received 15 October 1998; accepted in final form 17 December 1998.

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