Expanded CD14+ CD16+ Monocyte Subpopulation in Patients with Acute and Chronic Infections Undergoing Hemodialysis

WOLFGANG ANDREAS NOCKHER and JÜRGEN E. SCHERBERICH*

Institute of Clinical Chemistry, University Hospital Großhadern, Ludwig-Maximilians Universität München, and Second Medical Department, Hospital München-Harlaching, Munich, Germany

Received 24 October 1997/Returned for modification 21 January 1998/Accepted 31 March 1998

Infections are frequent complications in end-stage renal failure patients undergoing hemodialysis (HD), and peripheral blood monocytes are important cells in host defense against infections. The majority of circulating monocytes express high levels of lipopolysaccharide receptor antigen CD14 and are negative for the immunoglobulin Fc receptor type III (CD16). We studied the occurrence of a minor subpopulation coexpressing low levels of CD14 together with CD16 in HD patients. In healthy controls CD14+ CD16+ monocytes account for 8% ± 4% of CD14+ monocytes, with an absolute number of 29 ± 14 cells/µl. In stable HD patients the CD14+ CD16+ monocyte subpopulation was significantly elevated (14% ± 3%, or 66 ± 28 cells/µl), while the number of CD14+ monocytes (monocytes strongly positive for CD14) remained constant. In HD patients suffering from chronic infections a further rise in CD14+ CD16+ monocytes was observed (128 ± 71 cells/µl; P < 0.01) such that this subpopulation constituted 24% of all blood monocytes. In contrast, numbers of CD14+ cells did not change compared to those for stable HD patients, indicating that the CD14+ CD16+ monocyte subpopulation was selectively expanded. During acute infections the CD14+ CD16+ cell subpopulation always expanded. A whole-blood assay revealed that CD14+ CD16+ monocytes exhibited a higher phagocytosis rate for Escherichia coli bacteria than CD14+ monocytes, underlining their role during host defense. In addition, CD14+ CD16+ monocytes expressed higher levels of major histocompatibility complex (MHC) class II antigens (HLA-DR, -DP, and -DQ) and equal amounts of MHC class I antigens (HLA-ABC). Thus, CD14+ CD16+ cells constitute a potent phagocytosing and antigen-presenting monocyte subpopulation, which is expanded during acute and chronic infections commonly observed in chronic HD patients.

Peripheral blood monocytes are members of the mononuclear phagocytic system, which plays a central role in immunoregulation and host defense against immunopathogenic organisms (7). Monocytes are activated through molecular signals provided by structures of the infective organisms (8, 27, 28, 34, 35) or inflammatory mediators and chemotactic factors released by other cells during the infective challenge (22, 44, 47). However, blood monocytes represent a heterogeneous cell population and can be distinguished by variations in morphology (38, 58), membrane antigen expression (39), and release of inflammatory mediators (12, 25, 41).

While the lipopolysaccharide (LPS) receptor antigen CD14 is expressed by nearly all circulating peripheral blood monocytes, monocytes differ markedly in cell surface CD14 density as well as in the expression of immunoglobulin Fc receptors (53, 67). The majority of monocytes strongly positive for CD14 (CD14+) express Fcγ receptor I (CD64) and Fcγ receptor II (CD32) and are negative for Fcγ receptor III (CD16) (18). Only a small population was identified by the absence of Fcγ receptors (63). Nevertheless, a subset of monocytes characterized by low-level expression of CD14 and expression of the CD16 antigen has also been described (40). In healthy subjects these CD14+ CD16+ cells account for about 10% of all monocytes and are thought to be more mature cells than the regular CD14+ monocytes, as they exhibit features of tissue macrophages (66). In various infectious or inflammatory diseases such as AIDS and asthma the CD14+ CD16+ monocyte subpopulation is markedly expanded (36, 43, 50). A more than 10-fold increase of these cells during septicemia was demonstrated, and CD14+ CD16+ cells become the predominant type of monocytes in some septic patients (14).

Patients with end-stage renal failure undergoing chronic hemodialysis (HD) show an impaired immune response (10) with a high prevalence of infectious complications (17). Most of these infections are of bacterial origin, representing a major cause of morbidity and mortality in chronic HD patients (24). Furthermore, acute or chronic inflammatory processes, among them pneumonia and vascular access site infections, are common hazards in uremic patients undergoing chronic regular HD. Despite some data on the functional abnormalities of polymorphonuclear leukocytes in uremia (19), little information exists on the level of monocytes and their subsets in maintenance dialysis patients.

In an effort to further understand the importance of the distinct monocyte population expressing Fcγ receptor type III, we determined the levels of these cells in patients with end-stage renal failure undergoing chronic HD. This allowed the level of CD14+ CD16+ cells to be compared to that of CD14+ cells and the total monocyte count in whole blood. To investigate the proinflammatory role of CD14+ CD16+ monocytes, stable patients as well as patients with acute or chronic signs of infections or inflammatory processes were studied. Furthermore, we analyzed cell surface HLA expression of CD14+ CD16+ monocytes by immunophenotyping and compared their phagocytic competence with that of regular CD14+ blood monocytes.

MATERIALS AND METHODS

Patients. The patient population was divided into the following groups: informed outpatients on chronic maintenance HD with stable disease and no clinical or laboratory signs of an infectious episode (n = 18) and age- and...
Table 1. List of HD patients with chronic or recurrent infections

<table>
<thead>
<tr>
<th>No. of patients</th>
<th>Diagnosis(es)a</th>
<th>Clinical and laboratory dataa</th>
<th>Dialyzerb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>HCV, HBV</td>
<td>Elevated liver enzymes, icteric phases</td>
<td>PA</td>
</tr>
<tr>
<td>2</td>
<td>HIV</td>
<td>Fungal infections, chronic pneumonia, CRP↑</td>
<td>PA, HE</td>
</tr>
<tr>
<td>3</td>
<td>Diabetes mellitus</td>
<td>Keratoconjunctivitis, chronic bronchopneumonia, Klebsiella↑ +++, other coagulase↑, leukocytosis, CRP↑</td>
<td>PS, PA</td>
</tr>
<tr>
<td>1</td>
<td>Ophthalmia, arterial occlusive vasculopathy, dermatis perforans</td>
<td>Purulent infection, staphylococci↑++, anemia, leukocytosis</td>
<td>HE</td>
</tr>
<tr>
<td>1</td>
<td>Lymph node tuberculosis, urogenital tuberculosis</td>
<td>Chronic prostatitis, Mycobacterium hominis↑++, fever, anemia, CRP↑</td>
<td>PA</td>
</tr>
<tr>
<td>1</td>
<td>Atypical mycobacteriosis</td>
<td>Mycobacterial histiocytosis↑++, epithelial granuloma</td>
<td>PS</td>
</tr>
<tr>
<td>2</td>
<td>Unclear inflammatory disease</td>
<td>Recurrent fever, borderline leukocytosis, CRP↑</td>
<td>PS, HE</td>
</tr>
<tr>
<td>1</td>
<td>Irritable bowel, subileus</td>
<td>Leukocytosis</td>
<td>PS</td>
</tr>
<tr>
<td>2</td>
<td>Chronic pancreatitis</td>
<td>Elevated amylase and lipase activity, CRP↑</td>
<td>PA</td>
</tr>
<tr>
<td>1</td>
<td>Liver cirrhosis, chronic peritonitis</td>
<td>Recurrent diarrhea, pseudoperitonitis, leukocytosis, anemia</td>
<td>PA</td>
</tr>
<tr>
<td>1</td>
<td>AA-type amyloidosis</td>
<td>Irritable bowel, carpal tunnel syndrome, recurrent fever</td>
<td>PS</td>
</tr>
</tbody>
</table>

Note: a: HCV, hepatitis C virus; HBV, hepatitis B virus. b: CRP↑, CRP serum levels >20 mg/liter; leukocytosis, >10,800 cells/μl; borderline leukocytosis in HD patients, 9,000 to 10,500 cells/μl; +, positive pathogen determination; fever, temperature >38°C for more than 24 h. c: Dialyzer membranes: Polyamide (PA), Polysulfone (PS), and Hemophane (HE).

Sex-matched HD patients suffering from chronic infectious diseases (n = 16).

Among the patients monitored over a period of 6 months, those patients with recurrent infections were analyzed in more detail during the active infectious episodes. Such episodes were defined by one or more of the following conditions: increase in C-reactive protein (CRP), borderline leukocytosis and fever, leukocytosis, and of vascular access with fever and increase of acute-phase reactants, positive blood culture data, positive histological evaluation (e. g., tuberculosis), recurrent fever episodes due, e. g., to chronic bowel infection (subileus or pancreatitis), increase in serum amylase and lipase activity, recurrent fever due to underlying systemic disease (AA-type amyloidosis) or chronic bronchopneumonia (radiological signs of infiltrations), cirsosis of the liver and intermittent inflammatory signs, recurrent ophthalmitis, peripheral arterial occlusive disease with ulcers, and positive bacterial cultures of cutaneous smears. In addition, chronic viral infections (human immunodeficiency virus [HIV] or hepatitis A, B, or C) were monitored. Table 1 summarizes the diagnoses, as well as clinical and laboratory data, of the HD patients with chronic or recurrent infections studied.

All patients had been dialyzed three times a week for 4 to 5 h with the same dialysis membrane over the preceding 24 months. Dialyzers were not reused.

In 12 nonuremic patients who had undergone renal allotransplantation and who showed stable transplant function and no signs of inflammatory diseases were used as a second control group. Kidney transplant patients were under constant immunosuppressive therapy including low doses of steroids and cyclosporine; the latter was monitored to determine that its concentration was within the therapeutical range, i.e., between 80 and 150 ng/ml. Sixty-two volunteers recruited from our laboratory and clinical staff served as healthy controls.

Blood sampling. For counting blood monocytes and determining their cell surface antigen expression, whole blood was collected in tubes containing EDTA-Monovetten (Sarsted, Nümbrecht, Germany). Blood specimens were prepared for flow cytometry within 30 min after venipuncture. Absolute numbers of monocytes were calculated by using leukocyte counts derived from an automated blood cell counter (Coulter Electronics, Hialeah, Fla.).

Mabs and monococyte labelling. The following murine monoclonal antibody (Mab) clones used for phenotyping peripheral blood monocytes were purchased from Becton Dickinson (Heidelberg, Germany): Fluorescein isothiocyanate (FITC)-conjugated anti-CD14, phycoerythrin (PE)-conjugated anti-CD14, anti-CD16 (Fcγ receptor III), and PerCP-conjugated anti-CD45; these were purchased from Pharmingen (Hamburg, Germany) or the following: FITC-conjugated anti-CD16-AP, anti-HLA-DR, and anti-HLA-ABC that purchased from Immuno-techn (Hamburg, Germany) was PE/Cy5-conjugated anti-CD16. Fluorochrome-labelled anti-immunglobulin G1 (IgG1) and IgG2 isoctype control antibodies were purchased from Becton Dickinson.

For direct immunofluorescence labelling, 100 μl of whole blood was incubated with antigen-specific fluorochrome-labelled Mabs or the correspondent isotype control antibodies for 15 min at room temperature. For lysis of erythrocytes, 2 ml of lysis solution (Becton Dickinson) was added and the mixture was incubated for 10 min. Then cells were centrifuged, washed with phosphate-buffered saline, and fixed with 0.5 ml of 2% paraformaldehyde. Fixed cells were analyzed by flow cytometry within 6 h.

Flow cytometry analysis. Flow cytometric analysis was performed with a FACScan flow cytometer (Becton Dickinson). Monocytes were first gated according to their forward- and side-scatter profiles, and then FITC, PE, or PE/Cy5 channel fluorescence was measured within the monocyte gate. For the determination of specific antigen expression, the differences in fluorescence intensity between a specific Mab stain and the control stain were calculated. Results were expressed as mean fluorescence intensity (MFI).

The calibration of the flow cytometer was tested with Immuno-Check fluorospheres (Coulter-Immunotech) daily, and the instrument amplifier setting was adjusted with Immuno-Brite microbeads (Coulter-Immunotech) monthly.

Measurement of phagocytic activity. The quantitative determination of leucocyte phagocytosis in whole blood was performed with the Phagotest kit (Osthegen Pharma, Heidelberg, Germany). The percentages of monocytes and granulocytes showing phagocytosis as well as the amount of phagocytic activity per cell were determined. The performance of this test was modified in order to analyze different monocyte subpopulations according to their levels of membrane antigen expression. Briefly, for each specimen two test tubes were filled with 100 μl of heparinized blood and precooled on ice for 10 min before 20 μl of FITC-labelled Escherichia coli bacteria (2 × 10⁸ cells) was added. The control samples remained on ice, whereas assay samples were incubated for exactly 10 min at 37 ± 0.5°C in a water bath. At the end of incubation all samples were placed on ice, and 100 μl of quenching solution was added to suppress the fluorescence of adhering and noningested bacteria. After being washed with phosphate-buffered saline, monocytes were stained with a PE-conjugated anti-CD14 Mab and a PE/Cy5-conjugated anti-CD16 Mab for 15 min at 4°C. Then the whole blood was lysed, fixed with a lysis solution containing 1% paraformaldehyde as a fixative, and washed again.

Phagocytosis was monitored on a FACScan flow cytometer by three-color fluorescence measurement. Monocytes and granulocytes were first gated by their light scatter, and within the monocyte gate CD14⁺ and CD14⁻ CD16⁺ monocytes were further gated according to their levels of specific PE and PE/Cy5 fluorescence. Then the FITC fluorescence was determined for all cell populations; this represented the phagocytosis of FITC-labelled bacteria.

Within a relevant leukocyte cluster the FITC fluorescence levels of assay samples and control samples were compared to determine the percentages of...
RESULTS

Distribution of CD14+ CD16+ monocytes in healthy subjects and patients under HD. Leukocytes stained with anti-CD14 and anti-CD16 MAb revealed two subsets of peripheral blood monocytes in human blood (Fig. 1). Whereas nearly all monocytes were positive for the CD14 antigen, differences were found with regard to coexpression of the CD16 antigen. One population expressed high levels of CD14 (CD14+CD16−) and accounted for the majority (92% ± 4%) of CD14+ monocytes, with an absolute number of 336 ± 134 cells/µl in healthy donors (Table 2). The second population expressed lower levels of CD14 together with the CD16 antigen. These CD14+CD16+ monocytes constituted a minor population, i.e., about 8% ± 4% of CD14+ monocytes in healthy subjects (Fig. 1A), with an absolute number of 29 ± 14 cells/µl.

In patients undergoing chronic HD monocytes expressed significantly lower levels of LPS receptor antigen CD14, as was reported previously (37). The total monocyte counts in whole-blood specimens from stable HD patients without any signs of acute or chronic infections were significantly higher than those in specimens from healthy controls. Moreover, the distribution of monocyte subsets was altered (Table 2). The CD14+CD16− cell population was significantly elevated in stable HD patients compared to that in healthy controls. Also numbers of CD14+CD16− monocytes slightly increased, but this increase was not statistically significant. However, the percentage of CD14+CD16+ monocytes increased to 14% ± 3%, while the portion of CD14−CD16+ monocytes decreased in parallel.

HD patients with chronic or recurrent infections had an expanded population of CD14+CD16+ monocytes compared to stable HD patients. In these patients the CD14+CD16+ subset accounted for about 25% of all peripheral blood monocytes. In contrast, numbers of CD14+CD16− monocytes were equal in the two patient groups, indicating that especially the CD14+CD16+ monocyte population increased under these pathophysiological conditions (Table 2). Additionally, the total monocyte count increased only marginally compared to stable HD patients, and this rise was exclusively due to the expansion of the CD14+CD16− cell subpopulation. Therefore, a shift occurred in the blood monocyte pool from the CD14+CD16− to the CD14+CD16+ monocyte subset. This was confirmed by the

TABLE 2. Blood monocyte count and distribution of monocyte subpopulations

<table>
<thead>
<tr>
<th>Subject group</th>
<th>Count and distribution (P value) for:</th>
<th>Cells/µl</th>
<th>% Blood leukocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total monocytes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Healthy controls (n = 63)</td>
<td>366 ± 139</td>
<td>6.4 ± 1.5</td>
<td>336 ± 134</td>
</tr>
<tr>
<td>HD patients without infection (n = 18)</td>
<td>470 ± 171 (&lt;0.01)</td>
<td>7.4 ± 2.1 (&lt;0.05)</td>
<td>396 ± 151 (n.s.)</td>
</tr>
<tr>
<td>HD patients with chronic infection (n = 16)</td>
<td>515 ± 173 (n.s.)</td>
<td>7.9 ± 2.3 (n.s.)</td>
<td>392 ± 114 (n.s.)</td>
</tr>
</tbody>
</table>

* Whole-blood samples were stained simultaneously with anti-CD14 and anti-CD16 antibodies, and the percentages of CD14+CD16− and CD14+CD16+ cells were determined by fluorescence-activated cell sorter analysis within the monocyte gate. Absolute monocyte numbers were obtained by an automated leukocyte count, and the cell numbers of monocyte populations were calculated with the percentage distribution obtained from flow cytometry. Results are expressed as means ± SD, and statistical comparison between patient groups was carried out with the Student t test.

P values for HD patients without infection were obtained by comparison to healthy controls; those for HD patients with chronic infection were obtained by comparison to HD patients without infection.

n.s., not significant.
CD14$^+$ CD16$^+$ monocyte population seen in HD patients was found to be rather constant for individuals without acute infectious episodes. However, in the chronically infected group variations in the distribution of CD14$^+$ CD16$^-$ cells were greater than those seen in stable HD patients. This may be due to the fact that in the former group the definition and clinical presentation of acute, peracute, chronic relapsing, and chronic inflammatory changes may differ. In 12 patients who had undergone successful renal transplantation and who had normal graft function and stable clinical findings, the percentage of CD14$^+$ CD16$^-$ blood monocytes was $8.2\% \pm 2.2\%$, within the normal range. This observation suggests that, in the high-risk uremic population, the "chronic preinflammatory state" shows a certain recovery if a nonuremic situation is reached.

**Increase of the CD14$^+$ CD16$^+$ monocyte population during acute infections.** During phases of acute infectious episodes the CD14$^+$ CD16$^+$ monocyte subpopulation in HD patients was found to be markedly expanded (Fig. 4A), whereas changes in membrane CD14 density on peripheral blood monocytes were not uniform (Fig. 4B). However, changes in CD14 surface expression were parallel in CD14$^+$ and CD14$^-$ CD16$^+$ monocyte populations. In contrast, in six febrile HD patients (38.2 to 39.5°C) the increment of CD14$^+$ CD16$^+$ monocytes could be correlated with clinical and serological findings such as leukocytosis, increase of CRP, and positive blood culture. In four HD patients with acute illness, bacterial infections were found to be causative. In three cases staphylococci were involved (shunt infection, infected jugular vein catheter, infected right atrial catheter). Another patient suffered from acute tracheobronchitis (Klebsiella pneumoniae), fluid in the lung, and uveitis. Two other patients suffered either from acute cholangitis, enteritis, and subileus or acute bronchopneumonia (coughing, mucopurulent expectoration, lung infiltrations, respiratory alkalosis). In both cases blood cultures were positive for gram-negative bacteria. The first patient had a serum alkaline phosphatase level of 420 U/liter and a γ-glutamyl transpeptidase level of 267 U/liter; ultrasound investigation of the bowel disclosed a sludge phenomenon of the gall bladder and slightly enlarged intrahepatic bile ducts. The second patient had a homogeneous nonsegmental consolidation in the right and left lower lobes and small parapneumonic effusions of both sinus pleurae.

The increase in the number of CD14$^+$ CD16$^+$ cells during the active infectious challenge was statistically significant (14.6% ± 7% versus 32% ± 12%, $P < 0.001$). It is notable that an expansion of the CD14$^+$ CD16$^+$ subpopulation was found regardless of the baseline level of CD14$^+$ CD16$^+$ cells in individuals. Thus, even in patients with a large baseline CD14$^+$ CD16$^+$ population there was a further expansion during the acute infection as shown in Fig. 4. After initiation of adequate antibiotic therapy the decline of clinical symptoms was followed by a decrease of CD14$^+$ CD16$^+$ blood monocytes as demonstrated in more detail in the following two case reports.

Figure 5 concerns the case of a 50-year-old male outpatient undergoing chronic ambulatory peritoneal dialysis who was admitted to the hospital for removal of the peritoneal catheter and intermittent HD therapy in which a double-lumen jugular vein catheter (Shaldon) was used for blood access. After 2 days the skin adjacent to the inlet of the jugular vein catheter showed local heat and rubeosis and the patient developed fever (38°C), and mild leukocytosis. Blood cultures were positive for Staphylococcus aureus and Staphylococcus epidermidis. The CD14$^+$ CD16$^+$ monocyte population expanded from 13 to 28% of all CD14$^+$ monocytes during the acute infection, followed by a decline after antibiotic treatment with vancomycin. Cell surface CD14 density remained nearly con-
stant on CD14\textsuperscript{+} and CD14\textsuperscript{+} CD16\textsuperscript{+} monocytes (data not shown).

Another patient undergoing chronic intermittent HD (implanted right atrial venous catheter) was monitored for acute-phase reactant CRP, monocyte HLA-DR expression, and distribution of CD14\textsuperscript{+} CD16\textsuperscript{+} blood monocytes (Fig. 6). During the observation period the patient developed fever (39.8°C), general discomfort, and severe leukocytosis (18,000 cells/\textmu l). Vancomycin was administered together with imipenem/cilastatin. Meanwhile, a routine blood culture taken prior to the outbreak of clinical symptoms retrospectively disclosed an infection with enterococci. During the very early acute phase CD14\textsuperscript{+} CD16\textsuperscript{+} monocytes markedly increased, followed by a (later) decrease in monocyte HLA-DR expression. The altered monocyte phenotype pattern returned to normal together with the patient’s definite clinical improvement and recovery of serum CRP concentrations. Depressed HLA-DR expression by peripheral blood monocytes during acute and chronic infections has been previously described by our laboratory (45). Taken together, these results indicate that during acute infections an expansion of the CD14\textsuperscript{+} CD16\textsuperscript{+} blood monocyte subset occurs, followed by a decline after therapeutic intervention.

Phagocytic activities of CD14\textsuperscript{+} and CD14\textsuperscript{+} CD16\textsuperscript{+} blood monocytes. Phagocytosis of microorganisms or particles is one of the most important functions of phagocytic cells during an infective challenge. We therefore compared the phagocytic activities of granulocytes, CD14\textsuperscript{+} monocytes, and CD14\textsuperscript{+} CD16\textsuperscript{+} monocytes from 14 healthy donors by using opsonized FITC-labelled bacteria in a whole-blood assay.

No differences were found in the numbers of phagocytosing cells within the monocyte populations: 94\% ± 2\% of the
CD14+ monocytes and 92% ± 3% of the CD14+ CD16+ subpopulation were phagocytosis active. However, nearly all granulocytes were phagocytosing cells (98% ± 1%; P < 0.001 compared to CD14+ monocytes). As shown in Fig. 7, a quantitative determination of the phagocytic activity revealed differences between granulocytes and monocyte subsets. CD14+ CD16+ monocytes exhibited a higher level of E. coli FITC fluorescence, which correlates with the number of ingested bacteria, than CD14+ monocytes. In all tested blood specimens, the phagocytic activity of the CD14+ CD16+ monocyte population was significantly greater than that of CD14+ monocytes (MFI, 1,758 ± 368 versus 1,162 ± 131; P < 0.001), whereas granulocytes showed the highest phagocytosis rate (MFI, 2,253 ± 343; P < 0.001 compared to CD14+ CD16+ cells). These findings were uniform in all 14 blood specimens tested regardless of the absolute amount of phagocytosis measured within a probe. In Fig. 8 a quantitative determination of phagocytosis is shown for CD14+ and CD14+ CD16+ cells and granulocytes of individuals. It is notable that the phagocytic capacity of the individuals varied up to threefold. However, within a tested blood specimen CD14+ CD16+ cells showed greater phagocytic activity than CD14+ monocytes, while granulocytes were the most effective phagocytes.

**MHC antigen expression of CD14+ and CD14+ CD16+ cells.** The cell surface density of major histocompatibility complex (MHC) antigen expression by monocyte subpopulations was analyzed by three-color immunofluorescence. The results of the quantitative determination are displayed in Table 3. CD14+ CD16+ monocytes expressed significantly higher levels of MHC class II antigens. HLA-DR and HLA-DP expression was fourfold and about threefold higher, respectively, in these cells than in CD14+ monocytes. Also, the membrane expression of the HLA-DQ antigen was found to be increased in CD14+ CD16+ monocytes, but the difference in antigen density was not so pronounced. No difference in MHC class I expression was observed; CD14+ CD16+ and CD14+ monocytes expressed similar amounts of HLA-ABC antigens.

**DISCUSSION**

Several important functions during an infectious challenge, e.g., phagocytosis, intracellular killing of ingested microorganisms, and immunoregulatory activities, are performed by cells

![FIG. 7. Phagocytic activities of blood leukocyte populations. Phagocytosis of FITC-labelled E. coli bacteria was measured in a whole-blood assay by flow cytometry. Blood aliquots were either incubated at 0°C as controls or at 37°C to initiate phagocytosis. Monocytes and granulocytes were gated according their light scatter profiles, and monocyte subpopulations were determined by anti-CD14-PE and anti-CD16-PE/Cy5 immunofluorescence staining. Phagocytosis was determined by measuring E. coli FITC fluorescence, which correlates with the number of ingested bacteria. The figure shows phagocytosis rates of CD14+ monocytes, CD14+ CD16+ monocytes, and granulocytes from a representative example.](http://iai.asm.org/)

![FIG. 8. Comparison of leukocyte phagocytic activity in individuals. Phagocytosis of FITC-labelled E. coli bacteria in blood leukocytes from healthy donors was determined as indicated in the legend for Fig. 7. Data from 12 of 14 whole-blood specimens are shown. Leukocyte populations (CD14+ monocytes, CD14+ CD16+ monocytes, and granulocytes) from each donor are represented by the same symbol. Results are expressed as values of FITC MFI, which correlates with the number of bacteria ingested by each leukocyte population.](http://iai.asm.org/)
of the mononuclear phagocyte system. Therefore, different subsets of peripheral blood monocytes may play different and pivotal roles during the immune response. Recently, two subsets of peripheral blood monocytes with functional differences in bacterial uptake and antigen processing of Listeria monocytogenes were identified (64).

In the past many monocyte antigens that serve as receptors for recognition and processing of bacterial antigens have been described. The CD14 membrane antigen functions as a receptor for LPS from gram-negative bacteria (52, 56, 61) and triggers LPS-induced monocyte activation (29). Moreover, CD14 seems to be involved in the recognition of structures from gram-positive bacteria such as lipoteichoic acid and peptidoglycan (6, 59). In addition, receptors for the Fc region of IgG are important in the phagocytosis of IgG-opsonized microorganisms (53, 54). Fcγ receptor type III (CD16) exists in two polymorphic forms: the transmembrane form (FcγRIIIa) expressed in monocytes/macrophages and the glycosyl phosphatidylinositol-anchored form (FcγRIIIb) expressed in granulocytes (53, 54). The FcγRIIIa form on monocytes is reported to mediate important immunophysiological functions such as superoxide generation (51) and antibody-dependent cell mediated cytotoxicity (13), and signal transduction by Fcγ receptor type III is mediated through the γ chain of the Fcγ receptor type III complex (33, 60). It is known that immune complexes cross-link Fcγ receptors on the surfaces of monocytes and induce tumor necrosis factor secretion (9). Fcγ receptor type III has little affinity for monomeric IgG but binds polymeric IgG or immune complexes efficiently (5). Blockade of this receptor by an anti-CD16 MAb inhibits the clearance of opsonized cells in vivo, indicating the influence of this membrane receptor in the removal of immune complexes during infections (4).

While in the past peripheral blood monocytes were thought to be negative for CD16 and to upregulate this antigen during culture in vitro (5, 26), a minor subpopulation of circulating monocytes which express Fcɣ receptor type III together with smaller amounts of CD14 has been described (65). The CD14+ CD16+ monocyte subpopulation is significantly expanded in patients with severe infections (14) and can account for more than 50% of all peripheral blood monocytes during septicemia. In the present study the percentage of CD14+ CD16+ monocytes was found to be significantly higher in HD patients than in healthy controls, with a further increase in patients suffering from chronic infectious or inflammatory diseases. These findings further support the importance of the CD14+ CD16+ monocyte subpopulation during an infectious challenge.

It is well known that HD patients have an increased susceptibility to infectious diseases, and infections account for 30 to 40% of deaths in patients on long-term HD (32). These infections are generally due to common and not opportunistic pathogens (23), but mycobacterial infections and tuberculosis are also more frequent in HD patients than in healthy subjects (3, 21). The diagnosis of tuberculosis is often difficult to establish because the symptoms may mimic those of uremia (1), and thus infections with Mycobacterium tuberculosis with low-grade disease activity may be clinically symptomless. Recently, an elevated percentage (tripled compared to controls) of CD14+ CD16+ monocytes from pulmonary tuberculosis patients has been described (55), underlining the possible diagnostic value of the expanded CD14+ CD16+ cell population in HD patients with potential mycobacterial infections.

Viral diseases such as hepatitis B and C and cytomegalovirus infection are also common in dialysis patients; these contribute to the impaired immune response and increase the risk of further secondary infections. In addition, patients who receive dialysis therapy are under the continuous threat of infections from vascular or peritoneal access, which is clearly demonstrated by the case of the chronic ambulatory peritoneal dialysis patient described above. This issue has been discussed in detail by other investigators (48).

Recent studies reported expanded populations of CD14+ CD16+ monocytes in HIV-infected patients also prone to recurrent infectious crises (31, 36, 50). Together with the present observations for HD patients and those from other workers for sepsis patients (14), these studies show that the CD14+ CD16+ monocyte subpopulation plays an important role during an infectious challenge. This is consistent with the higher surface density of MHC class II molecules which are necessary for antigen presentation to T cells. There is growing evidence that the expression of each type of MHC class II molecule on human monocytes is regulated independently of the others (46, 62). Thus, increased expression of three different HLA class II clusters (DR, DQ, and DP antigens) by CD14+ CD16+ cells underlines their role as potent antigen-presenting cells. Furthermore, the cell surface densities of adhesion molecules such as LFA-1 and ICAM-1 known to be involved in leukocyte cell-cell interactions are higher in CD14+ CD16+ than in CD14+ CD16− monocytes (66).

Using PCR analysis Frankenberger et al. (15) showed that CD14+ CD16+ cells expressed levels of mRNA for the proinflammatory cytokines interleukin 1 (IL-1), IL-6, and tumor necrosis factor similar to those expressed by CD14+ CD16− monocytes. In contrast, the level of mRNA for the potent anti-inflammatory cytokine IL-10 (11, 49) was substantially lower or undetectable in CD14+ CD16+ cells (15). This altered cytokine expression together with the higher cell surface densities of MHC class II antigens indicates that CD14+ CD16+ monocytes represent a potent antigen-presenting and proinflammatory subpopulation, possibly triggering immune responses during an infectious challenge.

There are few parameters that can be useful for monitoring the host response and for predicting the progression of chronic and acute infections. The relevance of determinations of serum concentrations of inflammatory cytokines and other substances released during the activated immune response is still under investigation (2, 16). Monitoring the cellular immune system in patients with increased incidence of infections seems to be another important approach to develop strategies for clinical intervention (20, 42, 57). Here, peripheral blood monocytes and their HLA-DR expression have been studied (30). However, determination of the expression of the highly phagocytosing and antigen-presenting CD14+ CD16+ monocyte subset, which is expanded in various inflammatory and infectious diseases, may also be helpful.

ACKNOWLEDGMENTS

We thank H. W. L. Ziegler-Heitbrock, Institute of Immunology, University of Munich, for critical reading of the manuscript. The study was in part supported by a research grant of the Biotest Study Foundation, Dreieich, Germany.

The skillful assistance of Angelika Ruppert in the flow cytometric analyses is kindly appreciated.

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Editor: J. R. McGhee