Uncorrected Version. Published on July 26, 2005 as DOI:10.1189/jlb.0505251

Differential expression of function-related antigens on blood monocytes in children with hemolytic uremic syndrome

Gabriela C. Fernández,*,[1] María V. Ramos,* Sonia A. Gómez,* Graciela I. Dran,† Ramón Exeni,‡ Marta Alduncín,‡ Irene Grimoldi,‡ Graciela Vallejo,§ Christian Elías-Costa,§ Martín A. Isturiz,* and Marina S. Palermo*

*Divisions of *Immunology of the Institute of Hematological Investigations and †Experimental Medicine, Academia Nacional de Medicina and Nephrology Departments of the ‡Pediatric Municipal Hospital of San Justo and §Ricardo Gutiérrez Hospital, Buenos Aires, Argentina*

Abstract: Monocytes (Mo) mediate central functions in inflammation and immunity. Different subpopulations of Mo with distinct phenotype and functional properties have been described. Here, we investigate the phenotype and function of peripheral Mo from children with hemolytic uremic syndrome (HUS). For this purpose, blood samples from patients in the acute period of HUS (HUS AP) were obtained on admission before dialysis and/or transfusion. The Mo phenotypic characterization was performed on whole blood by flow cytometry, and markers associated to biological functions were selected: CD14 accounting for lipopolysaccharide (LPS) responsiveness, CD11b for adhesion, Fc receptor for immunoglobulin G type I (FcγRI)/CD64 for phagocytosis and cytotoxicity, and human leukocyte antigen (HLA)-DR for antigen presentation. Some of these functions were also determined. Moreover, the percentage of CD14+ CD16+ Mo was evaluated. We found that the entire HUS AP Mo population exhibited reduced CD14, CD64, and CD11b expression and decreased LPS-induced tumor necrosis factor production and Fcγ-dependent cytotoxicity. HUS AP showed an increased percentage of CD14+ CD16+ Mo with higher CD16 and lower CD14 levels compared with the same subset from healthy children. Moreover, the CD14++ CD16− Mo subpopulation of HUS AP had a decreased HLA-DR expression, which correlated with severity. In conclusion, the Mo population from HUS AP patients presents phenotypic and functional alterations. The contribution to the pathogenesis and the possible scenarios that led to these changes are discussed. *J. Leukoc. Biol.* 78: 000–000; 2005.

Key Words: *HUS · CD16 · CD14 · HLA-DR · TNF*

INTRODUCTION

The epidemic form of hemolytic uremic syndrome (HUS), the most common cause of acute renal failure in children, is characterized by hemolytic anemia, thrombocytopenia, and renal failure [1, 2]. HUS has been associated with enterohemorrhagic infections caused by Shiga toxin (Stx)-producing *Escherichia coli* organisms [3, 4]. Endothelial dysfunction appears to be an important factor in the sequence of events that leads to the microangiopathic process observed in HUS. Clinical and experimental evidence suggests that the inflammatory response plays a pivotal role in the pathophysiological mechanisms of HUS. In this regard, increased levels of monocyte (Mo)-derived cytokines are present in sera and urine of HUS patients [5, 6]. Moreover, Stx induces the synthesis of interleukin (IL)-1β, tumor necrosis factor (TNF), IL-6, and IL-8 in nonstimulated human Mo [7], and depletion of hepatic and splenic macrophages is able to modulate Stx cytotoxicity in mice [8]. These findings indicate that inflammatory mediators, locally or systemically produced by Stx-stimulated Mo/macrophages, may contribute to the development of HUS.

Mo are central elements in inflammation and immunity and mediate important functions such as phagocytosis, antigen presentation, and cytokine production. The expression of human leukocyte antigen (HLA)-DR is central in the processing and presentation of antigens and has been found to correlate highly with infection in many clinical scenarios [9]; the CD14 membrane antigen functions as a receptor for lipopolysaccharides (LPS) from gram-negative bacteria and triggers LPS-induced Mo activation [10–12]; type I receptors for the Fc region of immunoglobulin G (IgG; FcγRI or CD64) are important mediators of phagocytosis of IgG-opsonized microorganisms and cytotoxic mechanisms triggered by immune complexes [13–15]; and CD11b is involved in adhesion and migration of Mo across endothelium and in recruitment to inflamed tissues [16, 17].

Different subpopulations of Mo with distinct phenotypic and functional properties coexist in circulation. In healthy subjects, more than 80% of peripheral blood Mo strongly express the CD14 antigen and are negative for CD16 (CD14++ CD16−, regular Mo), whereas a minor population with weak expression of CD14 and positive for CD16 (CD14+ CD16+) accounts for

[1] Correspondence: Instituto de Investigaciones Hematológicas, Academia Nacional de Medicina, Pacheco de Melo 3081, C1425AUM, Buenos Aires, Argentina. E-mail: gfernandez@hematologia.anm.edu.ar

Received May 9, 2005; revised June 8, 2005; accepted June 16, 2005; doi: 10.1189/jlb.0505251.

less than 10% [18]. These CD14+ CD16+ Mo are considered to represent an activated, more mature subset with characteristics that resemble macrophages and dendritic cells [19–21]. Moreover, this subpopulation expands in various infectious and inflammatory diseases [22–27] and can account for more than 50% of all peripheral blood Mo during septicemia [23, 28].

The aim of the present study was to address the phenotype and functional properties of circulating Mo from HUS patients. The markers evaluated were selected based on their involvement in mediating important monocytic functions. Therefore, we investigated changes in the expression of CD14, CD64, CD11b, and HLA-DR and evaluated the impact of these changes on Mo functionality. Finally, we examined whether the CD14+ CD16+ subset of Mo occurred more often in these patients. For this purpose, we determined the percentage of CD14++ CD16– and CD14+ CD16+ Mo in HUS patients and analyzed the expression of cell-surface HLA-DR, CD14, and CD16 on this particular subset.

MATERIALS AND METHODS

Patients and samples

The study was approved by the Hospital Ethical Committees: "Comité de Bioética del Hospital Municipal del Niño de San Justo" (Buenos Aires, Argentina) and "Comité de Bioética del Hospital Ricardo Gutiérrez" (Capital Federal, Buenos Aires, Argentina). All patients were enrolled in this study after informed consent from their parents had been obtained. In total, 45 children in the acute period of HUS (HUS AP) were studied. The criteria for diagnosis were a microangiopathic hemolytic anemia with schizocytes, thrombocytopenia (platelet count $<150\times10^9$/L), and acute renal failure (serum creatinine level >106.0 μmol/L). All patients develop HUS after gastroenteritis consisting of bloody diarrhea. Sixty percent of the children were positive to Stx-producing *E. coli* O157, diagnosed by stool culture and/or presence of Stx antibody in serum. Twenty-two were girls, and 23 were boys. Clinical and biochemical data of patients are given in **Table 1**. Blood samples (2 ml) were obtained for biochemical and immunological studies by venopuncture into EDTA plastic tubes before dialysis and/or transfusion at different days from the time when diarrhea had started (onset diarrhea; Table 1). The amount of blood obtained from each patient was not enough to measure all the parameters evaluated in this study, and the number of patients and controls analyzed is indicated in each figure. Four children with HUS died within 15 days of admission, and 16 children also had a second sample of blood taken 1–6 month after clinical recovery (HUS after AP). Blood samples from three additional age- and sex-matched control groups were collected and processed identically: healthy children (HC) admitted for routine surgical procedures; acute uremic children (UC) with high serum urea (7.1–21.5 mmol/L) and creatinine (106.2–234.5 μmol/L) levels; these children suffered from acute, postinfectious glomerulonephritis, evidenced by the presence of a nephritic syndrome (proteinuria and hematuria), low complement levels, and good prognosis, and in some cases, neutrophilia was also observed in these patients; (3) infected children (IC) coursing an acute bacterial infection not related to HUS. Their peripheral white blood cell (WBC) count ranged between 10.6 and 29.5×10^9 cells/L with more than 60% of neutrophils. Platelet count, urea and creatinine levels, and hematocrit were within the normal range.

Antibodies and immunophenotypic studies

Measurement of CD14, CD64 (FcγRI), CD16 (FcγRIII), CD11b, and HLA-DR expression on Mo was performed by direct immunofluorescence flow cytometry using the following conjugated mouse anti-human monoclonal antibodies (mAb): CD14-Pc5 (IgG2a, Immunotech, Marseille, France), CD64-fluorescein isothiocyanate (FITC; IgG1, Immunotech), CD16-phycoerythrin (PE; R-PE, IgG1, BD Biosciences, San Jose, CA), CD11b-PE (IgG1, Immunotech), HLA DR-FITC (FITC, IgG2a, BD Biosciences). Whole blood samples (100 μl) were incubated with the specific, conjugated mAb for 30 min at room temperature. Then, erythrocytes were lysed using fluorescein-activated cell sorter lysing solution (BD Biosciences), washed, and resuspended in 0.2 μl ISOFLOW (International Link, S.A., Buenos Aires, Argentina). In all cases, isotype-matched antibodies were assayed in parallel, and the fluorescence was measured with a Becton Dickinson FACScan. The analysis was made on 100,000 events on each sample by using the Cell Quest program. Mo were identified and gated according to their forward- and light side-scattering (FSC/SSC) dot-plot profiles. Cells within this gate were >98% positive for CD14.

TABLE 1. Clinical and Biochemical Data of HUS Patients

	Severity of renal dysfunction[a]		
	Grade I	Grade II	Grade III
General parameters			
Age (months)	32 ± 27 (8–120)	18 ± 8 (13–36)	24 ± 26 (7–108)
Duration of diarrhea (days)	4.0 ± 2.8 (1–9)	5.0 ± 1.9 (3–8)	6.0 ± 3.0 (2–12)
Days on dialysis	0	3 ± 2 (0–6)	13 ± 7 (5–26)
Blood and renal parameters on admission			
Platelets ($\times10^9$/L)	69.0 ± 40.5 (29.7–167.2)	54.2 ± 71.6 (28.0–214.0)	48.6 ± 36.1 (16.5–150.3)
Hematocrit	25.0 ± 3.7 (21.0–34.6)	24.4 ± 3.9 (21.1–31.0)	28.9 ± 3.6 (24.0–37.1)
Urea (mmol/L)	17.1 ± 10.0 (6.8–46.0)	20.5 ± 9.6 (17.3–40.1)	24.6 ± 12.2 (8.2–52.2)
Creatinine (μmol/L)	195.0 ± 124.3 (61.9–513.0)	394.0 ± 150.4 (106.2–539.8)	420.1 ± 135.1 (160.9–610.0)
Days since onset of diarrhea[b]	3.0 ± 3.9 (1–15)	3.0 ± 1.2 (1–5)	3.5 ± 1.8 (1–8)

[a] According to Gianantonio's criteria [29], patients were classified as mild cases (Grade I: no anuria, n = 16), moderate cases (Grade II: <7 days of anuria, n = 11), or severe cases (Grade III: >7 days of anuria, n = 18). Data are presented as mean ± SD, and the range is shown between brackets. [b] Days after onset of diarrhea, where blood was obtained for the analyses reported.

Peripheral blood mononuclear cell (PBMC) isolation

Blood was diluted 1:2 with saline, layered on a Ficoll-Hypaque cushion (Ficoll Pharmacia, Uppsala, Sweden; Hypaque, Wintthrop Products, Buenos Aires, Argentina), and centrifuged at 400 *g* for 30 min as described previously [30]. PBMC were collected, washed twice, and resuspended in RPMI 10% heat-inactivated fetal calf serum and antibiotics. Viability of PBMC was >96%, as determined by the trypan blue exclusion test.

Influence of plasma on CD16, CD14, and CD64 expression of PBMC from healthy adult donors

PBMC (1.5×10^6 cells) from five different healthy adult donors were incubated in the presence of 20% HUS AP or control plasmas for 18 h at 37°C in 5% CO_2. Afterward, cells were washed twice and triple-stained with the specific mAb. The percentage and expression of the markers were determined by flow cytometry on Mo, identified according to their FSC/SSC properties and expression of CD14.

Modulation of CD64 expression by interferon-γ (IFN-γ)

PBMC (1×10^6) were incubated for 18 h in the presence or absence of 240 U/ml IFN-γ (Sigma Chemical Co., St. Louis, MO) at 37°C in 5% CO_2. After the incubation period, cells were washed and stained with anti-CD64- tandem conjugate and anti-CD14-PE (IgG1, Immunotech) antibodies. Flow cytometry was performed, and Mo were identified by their CD14 expression. Results were expressed as the ratio between the expression of CD64 with and without IFN-γ.

Reactive oxygen species (ROS)-dependent cytotoxicity triggered by immune complexes

ROS-dependent cytotoxicity was assayed using immune complexes formed with ovalbumin (OVA) and its specific rabbit IgG antibody (OVA-anti-OVA) as described previously [15, 31]. Briefly, 0.4×10^6 PBMC were incubated with 20 μg OVA-anti-OVA immune complexes and 2.5×10^5 unsensitized ^{51}Cr-labeled chicken erythrocytes. After incubation for 18 h at 37°C in 5% CO_2, the culture plate was centrifuged, and the radioactivity of supernatants and pellets was measured in a γ-counter. The mean release of ^{51}Cr in triplicate samples was expressed as the percentage of total radioactivity, after subtracting spontaneous release (in the absence of antibody), which was always less than 3%.

ROS generation

PBMC (0.6×10^6) were incubated with 1 mM dihydrorhodamine (DHR; Sigma Chemical Co.) at 37°C in 5% CO_2. After 15 min, phorbol 12-myristate 13-acetate (PMA; 20 ng/ml) was added and incubated for 30 additional min. DHR fluorescence was determined by flow cytometry.

TNF assay

PBMC (1×10^6) were incubated in the presence or absence (basal TNF production) of 1 μg/ml LPS (Sigma Chemical Co.) for 18 h at 37°C in 5% CO_2. After the incubation period, cells were centrifuged, and the supernatants were collected and stored at –20°C. Supernatant biological activity of TNF was determined using a sensitive actinomycin D-treated murine L929 fibroblast assay as described previously [32]. Briefly, 0.1 ml serial dilutions of Mo-derived supernatants were added to the L929 cell monolayer. After 18 h of incubation in the presence of 10 μg/ml actinomycin D (Amersham Biosciences, Piscataway, NJ), the plates were washed with phosphate-buffered saline, and viable cells were fixed and stained with crystal violet solution for 20 min at 37°C. Then, cells were solubilized using a 30% acetic acid solution. The resultant absorbance was measured with a microtiter plate reader (Organon Tecnika, Argentina) at 550 nm. Reciprocal of the dilution for 50% cytotoxicity [lytic unit 50 (LU_{50})] was used as TNF activity titer of each sample.

Statistical analysis

Results are expressed as the mean ± SE. Statistical analysis of the data was performed using a nonparametric unpaired Mann-Whitney test. *P* values less that 0.05 were considered significant. For evaluation of correlations between immunological and biochemical parameters, the nonparametric, two-tailed Spearman's rank correlation test was used.

RESULTS

Absolute count and percentage of circulating Mo in HUS children

Table 2 shows the WBC count, as well as the percentage and absolute count of Mo in all clinical groups included in this study. As can be appreciated, HUS AP and IC showed a marked leukocytosis. They also showed an increase in the percentage of Mo compared with HC, which was statistically significant only for IC. However, there was a significant increase in the absolute count of Mo in HUS AP and IC compared with HC. All these leukocyte parameters were restored completely after clinical recovery (HUS after AP). The absolute number and percentage of Mo in acute UC were comparable with the HC population.

Phenotypic characterization of peripheral Mo from HUS patients

We first analyzed the surface expression of several molecules that are critically involved in Mo functionality, considering the entire Mo population. We determined the expression of CD14, CD64 (FcγRI), CD11b, and HLA-DR on circulating Mo defined by light-scatter properties from HUS AP, HUS after AP, HC, IC, and UC. The results depicted in **Figure 1** indicate that Mo from HUS AP patients have a marked reduction in

TABLE 2. Absolute Number and Percentage (%) of Mo in the Different Clinical Groups

		HC (n=31)	HUS AP (n=45)	HUS after AP (n=19)	IC (n=21)	UC (n=28)
White blood cell count ($\times10^9$/L)	Mean ± SD	6.9 ± 2.3	18.3 ± 8.3*	8.6 ± 4.1†,‡	18.2 ± 9.3*	10.1 ± 4.6†,‡
	Median	6.7	16.2	7.8	16.3	8.5
	Range	3.4–12.5	2.8–52.2	3.6–21.0	6.1–51.2	3.2–20.9
Monocytes ($\times10^9$/L)	Mean ± SD	0.41 ± 0.22	1.06 ± 0.68*	0.35 ± 0.16†,‡	1.32 ± 0.80*	0.61 ± 0.27†,‡
	Median	0.36	0.92	0.30	1.14	0.56
	Range	0.09–0.87	0.17–3.36	0.15–0.66	0.44–3.78	0.10–1.11
% Monocytes	Mean ± SD	5.9 ± 2.3	7.4 ± 3.7	5.4 ± 2.2†	7.9 ± 2.6*	6.5 ± 2.7
	Median	5.7	6.9	5.4	7.5	5.9
	Range	2.4–11.5	2.1–25.0	2.2–10.2	4.3–13.4	3.1–13.0

* $P < 0.05$ versus HC; † $P < 0.05$ versus HUS AP; ‡ $P < 0.05$ versus IC.

CD14 expression compared only with HC and a reduced CD64 and CD11b expression compared with all other clinical groups. HLA-DR expression in HUS AP Mo was lower than in HC, but the difference was not statistically significant (data not shown).

Considering that IFN-γ is able to up-regulate CD64 in normal Mo [33], we incubated Mo from HUS and HC with IFN-γ. Up-regulation of CD64 induced by IFN-γ was more pronounced in Mo from HUS AP patients who had the lowest expression of this receptor to begin with. Moreover, the IFN-γ treatment led to an almost complete recovery of CD64 expression to the level observed for HC. The increase of CD64 induced by IFN-γ in HUS after AP was comparable with that one of HC [MFI of CD64 with IFN-γ/MFI of CD64 without IFN-γ: HC (n=10)=1.32±0.07*; HUS AP (n=13)=2.51±0.30; HUS after AP (n=6)=1.54±0.19; *P<0.05 vs. HUS AP].

Functional studies

We next studied two Mo functions that may be involved directly or indirectly in endothelial damage in HUS. We determined receptor-dependent and independent ROS generation and studied TNF production of Mo from HUS children and from the other clinical groups.

I. ROS generation

We determined the FcγR (CD64)-dependent cytotoxic potential of HUS AP Mo, measuring ROS-dependent cytotoxicity triggered by immune complexes. In line with the lower expression of CD64 found in HUS AP, CD64-dependent cytotoxicity was reduced significantly compared with HC; patients studied after the AP still showed a decreased cytotoxicity [percentage of cytotoxicity: HC (n=10)=46.2±5.1*; HUS AP (n=10)=27.4±2.5; HUS after AP (n=6)=29.3±4.8; *P<0.05 vs. HUS AP]. To evaluate whether the decreased ROS-dependent cytotoxicity of HUS AP Mo could be ascribed to an impairment of the ROS production mechanism, we determined ROS generation triggered by a direct protein kinase C (PKC)-activating stimulus (PMA) by flow cytometry. As shown in **Figure 2**, the percentage of ROS-producing Mo was significantly higher in HUS AP compared with HC and was still high after the AP (HUS after AP). As in UC and IC, the percentage of responding Mo showed an intermediate value between HUS AP and HC, and the differences were not statistically significant comparing these two groups. The level of ROS produced per cell, evaluated as the MFI, was similar in all clinical groups (data not shown).

II. Production of TNF

We assayed basal and LPS-stimulated production of TNF in Mo from the different clinical groups. We found that basal TNF production was similar in HUS AP Mo and HC Mo (**Fig. 3A**). Upon LPS stimulation, HUS AP Mo produced less TNF than HC (Fig. 3B), but the difference was not statistically significant. However, the increase in TNF production induced by LPS (Fig. 3C), calculated as the ratio between the TNF produced in the presence of LPS and the basal TNF production, revealed a lower capacity of HUS AP Mo to generate TNF in response to LPS compared with HC. Mo from HUS after AP showed the same pattern of TNF production as HUS AP. UC and IC Mo showed high basal TNF production (Fig. 3A) and failed to respond to LPS-increasing TNF levels (Fig. 3C).

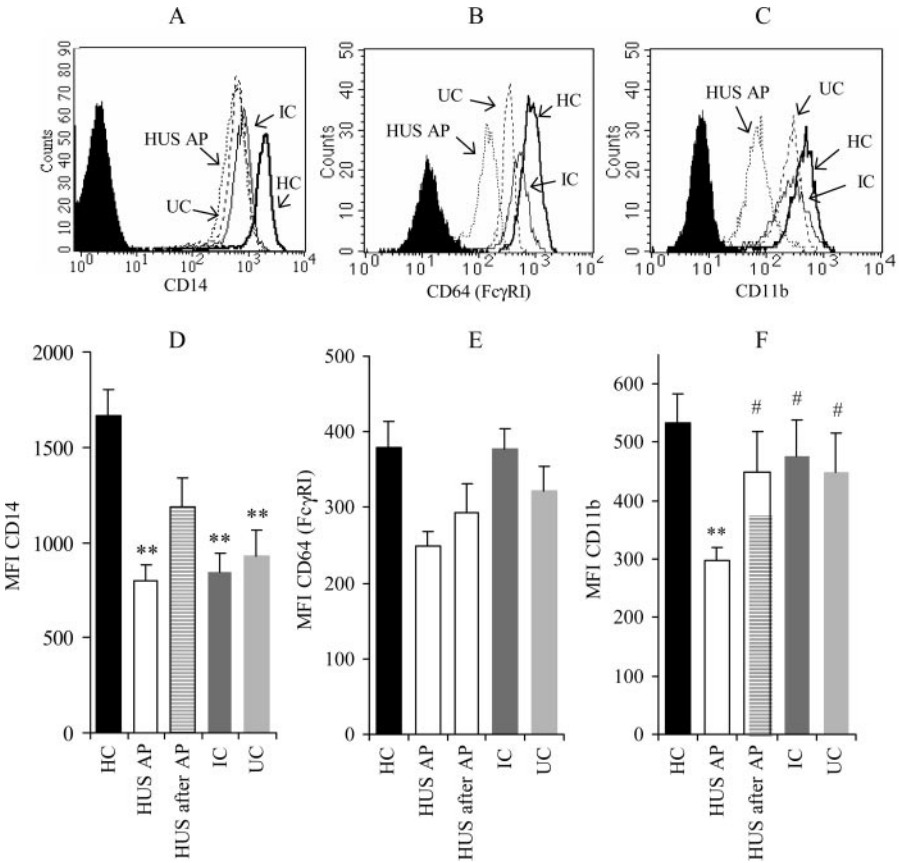

Fig. 1. Expression of CD14 (A, D), CD64 (B, E), and CD11b (C, F) on circulating Mo. The mean fluorescence intensity (MFI) of these markers was determined by flow cytometry as described in Materials and Methods. (Upper panels) Representative histograms showing the expression of CD14 (A), CD64 (B), and CD11b (C) of one child from each clinical group are shown. The filled histogram represents the isotype-matched control. (Lower panels) The graphs represent the mean ± SE of CD14 (D), CD64 (E), and CD11b (F) expression in HC (n=29), HUS AP (n=45), HUS after AP (n=16), IC (n=17), and UC (n=13). **, $P < 0.01$, versus HC; #, $P < 0.05$, versus HUS AP; ##, $P < 0.01$, versus HUS AP.

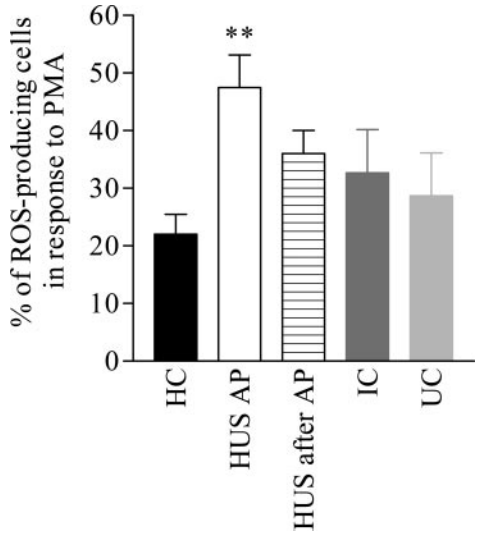

Fig. 2. PMA-induced ROS generation. Results are expressed as the mean ± SE of the percentage of Mo that produced ROS in response to PMA, measured as described in Materials and Methods, in HC (n=10), HUS AP (n=15), HUS after AP (n=5), IC (n=10), and UC (n=11). **, $P < 0.01$, versus HC.

Analysis of Mo subpopulations

Mo heterogeneity has been demonstrated recently using mAb and flow cytometry. Then, we further characterized the circulating Mo subpopulations in HUS patients, defined by three-color immunofluorescence using CD14, CD16, and HLA-DR antibodies. We distinguished the classical CD14++/CD16− cells and the CD14+ CD16+ Mo in HC, in similar percentages to those previously reported [18, 34]. However, as shown in **Figure 4B**, the percentage of CD14+ CD16+ Mo was significantly increased in HUS AP Mo and IC compared with HC. Although the entire HUS after AP group showed no differences in the percentage of CD14+ CD16+ Mo compared with HUS AP children (Fig. 4B), we found a strong negative correlation between the percentage of CD16+ Mo and the time elapsed since the AP (Fig. 4F). The expression of CD16 per cell was increased in HUS AP and IC Mo compared with HC but was unaffected in UC (Fig. 4C). We also analyzed the two major subsets of Mo (CD16− and CD16+) regarding the expression of CD14 and HLA-DR (Fig. 4, D and E, and **Fig. 5, B** and **C**, respectively). Consistent with previous reports, within the HC group, CD16+ Mo showed lower CD14 levels than CD16− Mo, but compared with HC, HUS AP Mo showed a significant decrease in CD14 expression, not only on the CD16+ subpopulation but also on the CD16− one (Fig. 4, D and E). A diminished expression of CD14 was also observed for IC and UC Mo (Fig. 4, D and E). Regarding HLA-DR expression (Fig. 5), CD16− Mo had a lower expression per cell of this antigen than the CD16+ population in all clinical groups. The expression of HLA-DR on the CD16− subset was decreased significantly in HUS AP Mo compared with HC and UC (Fig. 5B) but was similar for all clinical groups in the CD16+ subpopulation (Fig. 5C). The IC group also showed lower levels of HLA-DR in the CD16− subset.

Plasma influence

To determine the influence of circulating factors that can affect the expression of membrane markers of the Mo population, isolated PBMC from healthy adult donors were incubated with plasma from HUS AP patients or control group children. Figure 4G shows that HUS AP plasma induced a significant increase in the percentage of CD14+ CD16+ Mo in healthy adult donors compared with HC plasma. Plasma from IC also induced an increase in the percentage of CD16+ cells. Conversely, none of the plasmas assayed were able to modify the expression of CD16 per cell or the percentage or mean expression of CD14 and CD64 in healthy Mo (data not shown).

Correlations

Immunological, biochemical, and clinical data were correlated. We found a positive correlation between the percentage of CD16+ Mo and the duration (days) of diarrhea (Spearman r=0.40, P=0.03, n=28) and the levels of plasmatic urea (Spearman r=0.36, P=0.02, n=39). Moreover, a negative correlation was observed between the severity and the expression of HLA-DR, considering the entire Mo population (Spearman r=−0.48, P=0.03, n=22) or the CD16− subset solely (Spearman r=−0.70, P=0.005, n=16).

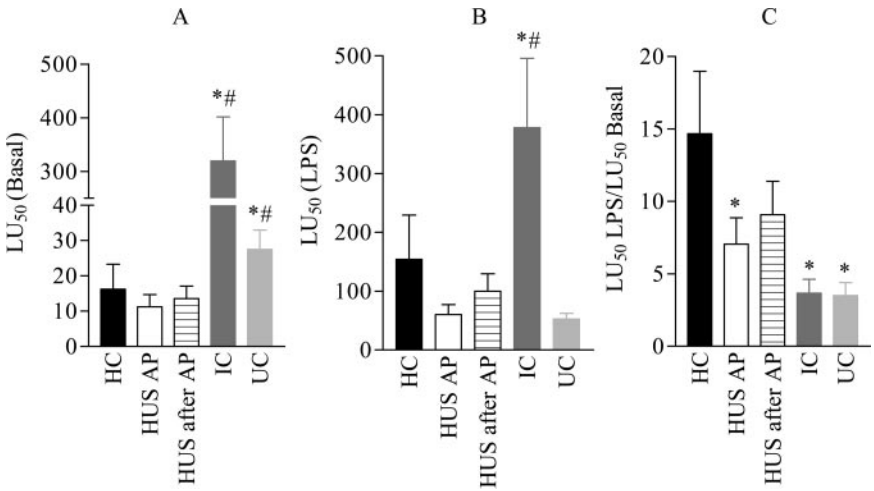

Fig. 3. Production of TNF by Mo. The TNF biological activity of Mo-derived supernatant was determined in HC (n=15), HUS AP (n=20), HUS after AP (n=13), IC (n=12), and UC (n=18), as described in Materials and Methods. Results are expressed as the mean ± SE of the LU_{50}, where the LU_{50} is defined as the reciprocal of the Mo-derived supernatant dilution that killed 50% of the L929 monolayer. (A) Basal TNF production. (B) LPS-induced TNF production. (C) LPS/basal ratio of TNF production. The increase in TNF production induced by LPS was calculated as the ratio between the LU_{50} in the presence of LPS with respect to the LU_{50} in the absence of LPS (basal TNF production). *, $P < 0.05$, versus HC; #, $P < 0.05$, versus HUS AP.

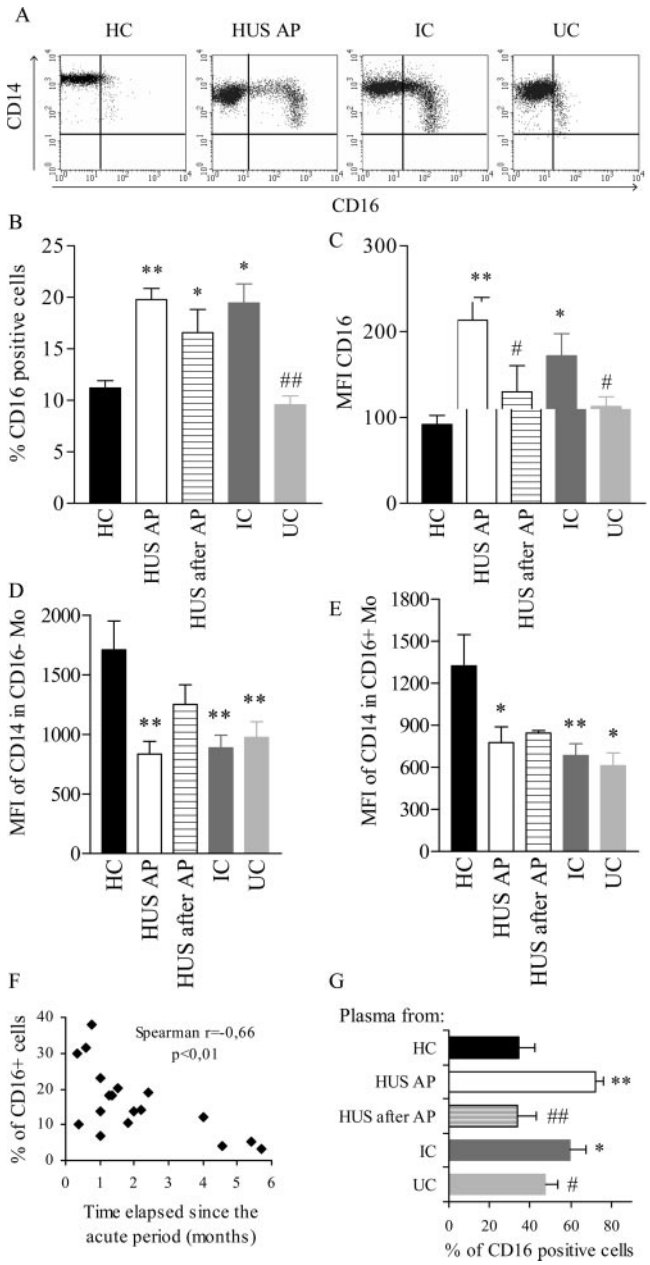

Fig. 4. Percentage of CD16+ Mo and analysis of the expression of CD14 and CD16 in Mo. Whole blood from HC (n=15), HUS AP (n=26), HUS after AP (n=5), IC (n=12), and UC (n=16) was stained with Pc5-labeled anti-CD14 and PE-labeled CD16 and analyzed by flow cytometry as described in Materials and Methods. Results are expressed as the mean ± SE. (A) Representative dot-plots of one child from each clinical group showing CD16 and CD14 expressions. (B) Percentage of CD16-positive cells in each group. (C) CD16 expression on the CD16+ Mo population. (D, E) CD14 expression on CD16− and CD16+ Mo, respectively. (F) Correlation between the percentage of CD16+ Mo in the HUS after AP group and the time elapsed (months) since the AP. (G) Induction of the CD16 antigen by plasma. PBMC from healthy adult donors were incubated with plasma from 10 different individuals of each clinical group in independent experiments. The percentage of CD16+ Mo after 18 h of plasma incubation was determined by flow cytometry as described in Materials and Methods, and the results are expressed as the mean ± SE of the percentage of CD16+ Mo. *, $P < 0.05$, versus HC; **, $P < 0.01$, versus HC; #, $P < 0.05$, versus HUS AP; ##, $P < 0.01$, versus HUS AP.

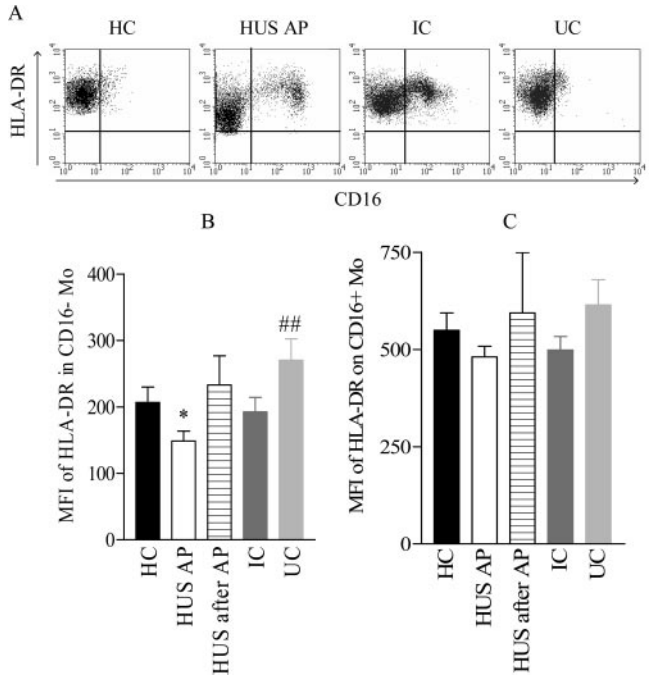

Fig. 5. Analysis of HLA-DR expression on Mo from HC (n=15), HUS AP (n=26), HUS after AP (n=5), IC (n=12), and UC (n=16). Whole blood was stained using PE-labeled CD16 and FITC-labeled HLA-DR and analyzed by flow cytometry as described in Materials and Methods. (A) Representative dot-plots of one child from each clinical group showing CD16 and HLA-DR expressions. (B, C) HLA-DR expression on CD16− and CD16+ Mo, respectively. Results are expressed as the mean ± SE of HLA-DR expression. *, $P < 0.05$, versus HC; ##, $P < 0.01$, versus HUS AP.

DISCUSSION

Mononuclear phagocytes play a central role in the immune response by presenting antigens and producing cytokines, thus initiating and regulating innate and adaptive immune responses. Whereas most studies in HUS patients have focused on various aspects of cytokine secretion in vivo, information about the expression of cell-surface antigens and their correlation with Mo activation is lacking. Our study revealed profound alterations in the Mo population from HUS patients. The whole monocytic population of HUS children showed decreased levels of CD14, CD64 (FcγRI), and CD11b expression. The decrease of CD14 membrane expression on Mo has been associated with LPS stimulation in in vitro and in vivo conditions [35–37]. Although endotoxemia has not been reported in HUS patients, LPS stimulation of the immune system in this disease has been vastly suggested [3]. The reduced expression observed for CD64 and CD11b on the entire HUS Mo population is a hallmark observation, as the other clinical groups neither share this abnormality nor has it been described previously in other pathological conditions. Enzymatic cleavage of cell membrane molecules by proteases released from activated cells (i.e., Mo and neutrophils) is an important mechanism of membrane antigen down-modulation [37, 38] and can account for the decreased expression of the Mo membrane antigens mentioned above. In this regard, degranulation of neutrophils [39, 40] and neutrophil-derived proteases in serum have been found in HUS patients [41]. Moreover, the reduced expression

of the membrane receptors CD64 and CD14 is probably associated with the functional impairment observed in Mo from HUS AP patients. In fact, ROS-dependent cytotoxicity triggered by immune complexes was found diminished, although the ROS generation in response to PMA was even higher in HUS AP children compared with HC. This can be explained, as ROS production triggered by PMA involves a receptor-independent pathway in which PKC is activated directly, whereas ROS production, triggered by immune complexes, is dependent mainly on CD64, the high-affinity receptor for IgG. These facts indicate that the impaired ROS-dependent cytotoxicity may be related to the decreased CD64 expression rather than to a deficiency in the ROS-producing machinery (the reduced nicotinamide adenine dinucleotide phosphate-oxidase). Although CD64 levels were restored by IFN-γ, further studies are necessary to determine whether immune complex-triggered ROS production is concomitantly recovered after IFN-γ treatment. Additionally, HUS AP Mo showed a decreased in vitro TNF production in response to LPS, which is probably a consequence of the low CD14 expression observed in these patients. In line with these findings, several reports have described a reduced ability to produce TNF in response to LPS in Mo from patients with sepsis and trauma and reported that this phenomenon of Mo deactivation shows similarities to experimental monocytic refractoriness induced by LPS desensitization [42, 43]. Similarly, IC and UC also showed a decreased CD14 expression and failed to respond to LPS by secreting TNF. On the contrary, these two control groups evidenced a higher TNF production in the absence of any stimulus (basal production), suggesting a different in vivo context at the moment of sample collection.

β2-integrins (CD18/CD11b) on myeloid cells were initially identified as cell-surface structures mediating the traffic and localization of circulating immune cells [44], but they are also able to regulate multiple cellular responses to environmental stimuli [45]. Although we have not directly assayed any CD11b-dependent function in HUS AP Mo, we can speculate that their reduced CD11b surface expression would lead to alterations in the trafficking and adherence properties to inflamed endothelium. In this regard, the down-regulation of membrane molecules may represent a mechanism to counteract ongoing inflammation and limit Mo adhesion and/or activation.

Regarding Mo subpopulations, we found a significant increase in the percentage of CD14+ CD16+ Mo in HUS AP patients and IC. This observation, together with studies reporting an expansion of this subpopulation in several infectious and inflammatory diseases, indicates that the increase of CD14+ CD16+ Mo may be associated with a strong activation of the immune system caused by an infectious challenge. It is known that CD16+ Mo have lower expression of CD14 and higher expression of HLA-DR antigen than CD16− Mo [34]. Although our data clearly support these observations for the HC group, in HUS AP patients, the CD14 expression was similarly low on CD16+ and CD16− Mo and in both subsets, was significantly decreased with respect to the HC group. Conversely, the expression of HLA-DR on CD16+ Mo from HUS AP children was higher compared with CD16− Mo and was similar to HC. However, CD16− Mo from HUS AP showed a significantly decreased HLA-DR expression compared with the same subset from HC. Several investigators have reported low levels of HLA-DR in patients after major surgery, severe trauma, and sepsis, which usually correlated with poor clinical outcome [46–49]. In keeping with these observations, we found an inverse correlation between Mo HLA-DR expression on admission and the severity in the HUS AP group. It is interesting that the CD14+ CD16+ subset present in HUS AP children exhibited a high expression of CD16, a similar feature found in the IC population and in septic neonates [50].

After clinical recovery, several of the parameters evaluated in the HUS after AP group (CD14 and CD64 expression, ROS generation, LPS-induced TNF production, and percentage of CD16-positive cells) presented intermediate values between HC and HUS AP children, and therefore, the differences were not statistically significant upon comparing these two groups. Although the time elapsed since the AP is heterogeneous, most of the samples were obtained between 15 days and 2 months after the AP. Several reports have suggested that 1–3 months after the AP of HUS, the environment of circulating cells may not be restored completely, and a local and persistent, inflammatory/anti-inflammatory reaction (i.e., at the renal endothelial level) may still be occurring [39, 40, 51]. However, as can be appreciated for the percentage of CD16+ cells in the clinically recovered children, the resume of this antigen to normal values correlated with the time elapsed since the AP (Fig. 4G), and patients studied after 3 months showed normal percentages of CD16+ cells. It would be interesting to evaluate the activation state of Mo from HUS patients at least 6 months after the AP to ascertain whether this long-term recovery applies to other antigens and functions.

What drives an expansion of the CD14+ CD16+ subset in vivo is still unknown, but cytokines such as IL-10 and transforming growth factor-β1 can favor the differentiation of CD16− into CD16+ Mo [20, 42, 52, 53]. The fact that these two cytokines have been found elevated in the plasma of HUS patients [5, 54, 55], together with the induction of the CD16 antigen in Mo from healthy adults incubated with HUS AP and IC plasmas, supports the involvement of soluble mediators in the expansion of the CD14+ CD16+ subset. However, whether the pattern of cytokines found in the plasma from HUS children is a cause (precedes) or a consequence (follows) of the imbalanced Mo subpopulations needs further investigation.

Whether the CD14+ CD16+ subset plays a beneficial or detrimental role in the fate of HUS remains elusive. Originally referred as "proinflammatory Mo", based on their relative high capacity to produce inflammatory cytokines, these Mo were implicated in the elevated levels of plasmatic inflammatory mediators observed in infectious diseases [56], and it was suggested that the determination of CD14+ CD16+ Mo levels could be useful for monitoring adult patients with high risk of sepsis [57]. Moreover, therapeutic approaches that reduce immune activation and prevent severe complications of inflammatory diseases, such as intravenous γ globulin or glucocorticoid treatments, rapidly decrease the CD14+ CD16+ Mo subpopulation [25, 58]. It has been reported that the CD16+ subpopulation of Mo exhibits higher levels of very late antigen 4, the ligand for vascular adhesion molecule-1, and CD11a and -c, which bind to intercellular adhesion molecule-1, so these cells may have a greater tendency to adhere to endothelium

and to migrate to tissues [19, 25]. These observations, in addition to the proinflammatory properties attributed to this subset, support the idea that CD14+ CD16+ Mo could contribute to HUS pathogenesis by enhancing endothelial injury. However, an anti-inflammatory role of CD16+ Mo has also been proposed [50, 52, 59]. In this sense, IL-10 production has been ascribed to the CD16+ subset [50, 59], and in some diseases, the high plasma levels of IL-10 found have been correlated with the elevated proportion of CD14+ CD16+ Mo [25, 50]. Considering all these issues, our next purpose will be to study the profile of cytokines produced by the different Mo subsets from HUS patients to clarify their role in the development and/or resolution of HUS.

In summary, this study describes phenotypic and functional changes of peripheral Mo in the AP of HUS. Monitoring the cellular immune system in patients with infections seems to be an important approach to develop strategies for clinical intervention. Besides, finding early markers that reflect the clinical outcome, as the correlation between HLA-DR and severity, can be useful and may have important therapeutic implications.

ACKNOWLEDGMENTS

This investigation was supported by an "R. Carrillo-A. Oñativia" award from Ministerio de Salud de la Nación, by Fundación "Alberto J. Roemmers," and by Agencia Nacional de Promoción Científica y Tecnológica, Argentina. The authors thank Marta Felippo, Nora Galassi, and Norma Riera for their excellent technical assistance and Fabiana Alberto and Emilse Bermejo for healthy adult blood samples. The authors also thank Fundación de la Hemofilia and Academia Nacional de Medicina for the use of the FACScan flow cytometer.

REFERENCES

1. Karmali, M. A., Petric, M., Lim, C., Fleming, P. C., Arbus, G. S., Lior, H. (1985) The association between idiopathic hemolytic uremic syndrome and infection by verotoxin-producing *Escherichia coli*. *J. Infect. Dis.* **151,** 775–782.
2. Remuzzi, G., Ruggenenti, P. (1995) The hemolytic uremic syndrome. *Kidney Int.* **48,** 2–19.
3. Paton, J. C., Paton, A. W. (1998) Pathogenesis and diagnosis of Shiga toxin-producing *Escherichia coli* infections. *Clin. Microbiol. Rev.* **11,** 450–479.
4. Keusch, G. T., Acheson, D. W. (1997) Thrombotic thrombocytopenic purpura associated with Shiga toxins. *Semin. Hematol.* **34,** 106–116.
5. Litalien, C., Proulx, F., Mariscalco, M. M., Robitaille, P., Turgeon, J. P., Orrbine, E., Rowe, P. C., McLaine, P. N., Seidman, E. (1999) Circulating inflammatory cytokine levels in hemolytic uremic syndrome. *Pediatr. Nephrol.* **13,** 840–845.
6. Proulx, F., Turgeon, J. P., Litalien, C., Mariscalco, M. M., Robitaille, P., Seidman, E. (1998) Inflammatory mediators in *Escherichia coli* O157:H7 hemorrhagic colitis and hemolytic-uremic syndrome. *Pediatr. Infect. Dis. J.* **17,** 899–904.
7. van Setten, P. A., Monnens, L. A., Verstraten, R. G., van den Heuvel, L. P., van Hinsbergh, V. W. (1996) Effects of verocytotoxin-1 on nonadherent human monocytes: binding characteristics, protein synthesis, and induction of cytokine release. *Blood* **88,** 174–183.
8. Palermo, M. S., Alves Rosa, M. F., Van Rooijen, N., Isturiz, M. A. (1999) Depletion of liver and splenic macrophages reduces the lethality of Shiga toxin-2 in a mouse model. *Clin. Exp. Immunol.* **116,** 462–467.
9. Cheadle, W. G. (1993) The human leukocyte antigens and their relationship to infection. *Am. J. Surg.* **165,** 75S–81S.
10. Troelstra, A., Antal-Szalmas, P., de Graaf-Miltenburg, L. A., Weersink, A. J., Verhoef, J., Van Kessel, K. P., Van Strijp, J. A. (1997) Saturable CD14-dependent binding of fluorescein-labeled lipopolysaccharide to human monocytes. *Infect. Immun.* **65,** 2272–2277.
11. Viriyakosol, S., Kirkland, T. N. (1996) The N-terminal half of membrane CD14 is a functional cellular lipopolysaccharide receptor. *Infect. Immun.* **64,** 653–656.
12. Liu, M. K., Herrera-Velit, P., Brownsey, R. W., Reiner, N. E. (1994) CD14-dependent activation of protein kinase C and mitogen-activated protein kinases (p42 and p44) in human monocytes treated with bacterial lipopolysaccharide. *J. Immunol.* **153,** 2642–2652.
13. van de Winkel, J. G., Anderson, C. L. (1991) Biology of human immunoglobulin G Fc receptors. *J. Leukoc. Biol.* **49,** 511–524.
14. van de Winkel, J. G., Capel, P. J. (1993) Human IgG Fc receptor heterogeneity: molecular aspects and clinical implications. *Immunol. Today* **14,** 215–221.
15. Geffner, J. R., Giordano, M., Serebrinsky, G., Isturiz, M. (1987) The role of reactive oxygen intermediates in nonspecific monocyte cytotoxicity induced by immune complexes. *Clin. Exp. Immunol.* **67,** 646–654.
16. Meerschaert, J., Furie, M. B. (1995) The adhesion molecules used by monocytes for migration across endothelium include CD11a/CD18, CD11b/CD18, and VLA-4 on monocytes and ICAM-1, VCAM-1, and other ligands on endothelium. *J. Immunol.* **154,** 4099–4112.
17. Patarroyo, M. (1994) Adhesion molecules mediating recruitment of monocytes to inflamed tissue. *Immunobiology* **191,** 474–477.
18. Ziegler-Heitbrock, H. W. (1996) Heterogeneity of human blood monocytes: the CD14+ CD16+ subpopulation. *Immunol. Today* **17,** 424–428.
19. Ziegler-Heitbrock, H. W., Fingerle, G., Strobel, M., Schraut, W., Stelter, F., Schutt, C., Passlick, B., Pforte, A. (1993) The novel subset of CD14+/CD16+ blood monocytes exhibits features of tissue macrophages. *Eur. J. Immunol.* **23,** 2053–2058.
20. Ancuta, P., Weiss, L., Haeffner-Cavaillon, N. (2000) CD14+CD16++ cells derived in vitro from peripheral blood monocytes exhibit phenotypic and functional dendritic cell-like characteristics. *Eur. J. Immunol.* **30,** 1872–1883.
21. Thomas, R., Lipsky, P. E. (1994) Human peripheral blood dendritic cell subsets. Isolation and characterization of precursor and mature antigen-presenting cells. *J. Immunol.* **153,** 4016–4028.
22. Nockher, W. A., Scherberich, J. E. (1998) Expanded CD14+ CD16+ monocyte subpopulation in patients with acute and chronic infections undergoing hemodialysis. *Infect. Immun.* **66,** 2782–2790.
23. Fingerle, G., Pforte, A., Passlick, B., Blumenstein, M., Strobel, M., Ziegler-Heitbrock, H. W. (1993) The novel subset of CD14+/CD16+ blood monocytes is expanded in sepsis patients. *Blood* **82,** 3170–3176.
24. Kawanaka, N., Yamamura, M., Aita, T., Morita, Y., Okamoto, A., Kawashima, M., Iwahashi, M., Ueno, A., Ohmoto, Y., Makino, H. (2002) CD14+,CD16+ blood monocytes and joint inflammation in rheumatoid arthritis. *Arthritis Rheum.* **46,** 2578–2586.
25. Katayama, K., Matsubara, T., Fujiwara, M., Koga, M., Furukawa, S. (2000) CD14+CD16+ monocyte subpopulation in Kawasaki disease. *Clin. Exp. Immunol.* **121,** 566–570.
26. Nockher, W. A., Bergmann, L., Scherberich, J. E. (1994) Increased soluble CD14 serum levels and altered CD14 expression of peripheral blood monocytes in HIV-infected patients. *Clin. Exp. Immunol.* **98,** 369–374.
27. Thieblemont, N., Weiss, L., Sadeghi, H. M., Estcourt, C., Haeffner-Cavaillon, N. (1995) CD14lowCD16high: a cytokine-producing monocyte subset which expands during human immunodeficiency virus infection. *Eur. J. Immunol.* **25,** 3418–3424.
28. Blumenstein, M., Boekstegers, P., Fraunberger, P., Andreesen, R., Ziegler-Heitbrock, H. W., Fingerle-Rowson, G. (1997) Cytokine production precedes the expansion of CD14+CD16+ monocytes in human sepsis: a case report of a patient with self-induced septicemia. *Shock* **8,** 73–75.
29. Gianantonio, C. A., Vitacco, M., Mendilaharzu, F., Gallo, G. E., Sojo, E. T. (1973) The hemolytic-uremic syndrome. *Nephron* **11,** 174–192.
30. Boyum, A. (1968) Separation of leukocytes from blood and bone marrow. Introduction. *Scand. J. Clin. Lab. Invest. Suppl.* **97,** 7.
31. Isturiz, M. A., Giordano, M., Serebrinsky, G. P. (1983) Effect of rheumatoid factors and normal human sera on immune complex-Fc γ R interaction. *Clin. Exp. Immunol.* **54,** 225–231.
32. Wang, A. M., Creasey, A. A., Ladner, M. B., Lin, L. S., Strickler, J., Van Arsdell, J. N., Yamamoto, R., Mark, D. F. (1985) Molecular cloning of the complementary DNA for human tumor necrosis factor. *Science* **228,** 149–154.
33. Comber, P. G., Lentz, V., Schreiber, A. D. (1992) Modulation of the transcriptional rate of Fc γ receptor mRNA in human mononuclear phagocytes. *Cell. Immunol.* **145,** 324–338.

34. Passlick, B., Flieger, D., Ziegler-Heitbrock, H. W. (1989) Identification and characterization of a novel monocyte subpopulation in human peripheral blood. *Blood* **74,** 2527–2534.
35. Nockher, W. A., Scherberich, J. E. (1995) Monocyte cell-surface CD14 expression and soluble CD14 antigen in hemodialysis: evidence for chronic exposure to LPS. *Kidney Int.* **48,** 1469–1476.
36. Hiki, N., Berger, D., Prigl, C., Boelke, E., Wiedeck, H., Seidelmann, M., Staib, L., Kaminishi, M., Oohara, T., Beger, H. G. (1998) Endotoxin binding and elimination by monocytes: secretion of soluble CD14 represents an inducible mechanism counteracting reduced expression of membrane CD14 in patients with sepsis and in a patient with paroxysmal nocturnal hemoglobinuria. *Infect. Immun.* **66,** 1135–1141.
37. Bazil, V., Strominger, J. L. (1991) Shedding as a mechanism of down-modulation of CD14 on stimulated human monocytes. *J. Immunol.* **147,** 1567–1574.
38. Beigier-Bompadre, M., Barrionuevo, P., Alves-Rosa, F., Rubel, C. J., Palermo, M. S., Isturiz, M. A. (2003) The formyl peptide N-formyl-methionyl-leucyl-phenylalanine downregulates the expression of FcγRs in interferon-γ-activated monocytes/macrophages in vitro and in vivo. *Scand. J. Immunol.* **57,** 221–228.
39. Fernandez, G., Gómez, S. A., Rubel, C., Bentancor, L. V., Barrionuevo, P., Alduncín, M., Grimoldi, I., Exeni, R., Isturiz, M. A., Palermo, M. S. (2005) Impaired neutrophils (PMN) in children with the typical form of hemolytic uremic syndrome (D+HUS). *Pediatr. Nephrol.*, Epub ahead of print.
40. Fernandez, G. C., Rubel, C., Barrionuevo, P., Lopez, L., Ramirez, F., Diaz, M., Isturiz, M. A., Palermo, M. S. (2002) Phenotype markers and function of neutrophils in children with hemolytic uremic syndrome. *Pediatr. Nephrol.* **17,** 337–344.
41. Kaplan, B. S., Mills, M. (1988) Elevated serum elastase and α-1-antitrypsin levels in hemolytic uremic syndrome. *Clin. Nephrol.* **30,** 193–196.
42. Docke, W. D., Randow, F., Syrbe, U., Krausch, D., Asadullah, K., Reinke, P., Volk, H. D., Kox, W. (1997) Monocyte deactivation in septic patients: restoration by IFN-γ treatment. *Nat. Med.* **3,** 678–681.
43. Wilhelm, W., Grundmann, U., Rensing, H., Werth, M., Langemeyer, J., Stracke, C., Dhingra, D., Bauer, M. (2002) Monocyte deactivation in severe human sepsis or following cardiopulmonary bypass. *Shock* **17,** 354–360.
44. Springer, T. A. (1994) Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. *Cell* **76,** 301–314.
45. Hynes, R. O. (1992) Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* **69,** 11–25.
46. Ditschkowski, M., Kreuzfelder, E., Rebmann, V., Ferencik, S., Majetschak, M., Schmid, E. N., Obertacke, U., Hirche, H., Schade, U. F., Grosse-Wilde, H. (1999) HLA-DR expression and soluble HLA-DR levels in septic patients after trauma. *Ann. Surg.* **229,** 246–254.
47. Hershman, M. J., Cheadle, W. G., Wellhausen, S. R., Davidson, P. F., Polk Jr., H. C. (1990) Monocyte HLA-DR antigen expression characterizes clinical outcome in the trauma patient. *Br. J. Surg.* **77,** 204–207.
48. Wakefield, C. H., Carey, P. D., Foulds, S., Monson, J. R., Guillou, P. J. (1993) Changes in major histocompatibility complex class II expression in monocytes and T cells of patients developing infection after surgery. *Br. J. Surg.* **80,** 205–209.
49. van den Berk, J. M., Oldenburger, R. H., van den Berg, A. P., Klompmaker, I. J., Mesander, G., van Son, W. J., van der Bij, W., Sloof, M. J., The, T. H. (1997) Low HLA-DR expression on monocytes as a prognostic marker for bacterial sepsis after liver transplantation. *Transplantation* **63,** 1846–1848.
50. Skrzeczynska, J., Kobylarz, K., Hartwich, Z., Zembala, M., Pryjma, J. (2002) CD14+CD16+ monocytes in the course of sepsis in neonates and small children: monitoring and functional studies. *Scand. J. Immunol.* **55,** 629–638.
51. Sassetti, B., Vizcarguenaga, M. I., Zanaro, N. L., Silva, M. V., Kordich, L., Florentini, L., Diaz, M., Vitacco, M., Sanchez Avalos, J. C. (1999) Hemolytic uremic syndrome in children: platelet aggregation and membrane glycoproteins. *J. Pediatr. Hematol. Oncol.* **21,** 123–128.
52. Olikowsky, T., Wang, Z. Q., Dudhane, A., Horowitz, H., Conti, B., Hoffmann, M. K. (1997) Two distinct pathways of human macrophage differentiation are mediated by interferon-γ and interleukin-10. *Immunology* **91,** 104–108.
53. Calzada-Wack, J. C., Frankenberger, M., Ziegler-Heitbrock, H. W. (1996) Interleukin-10 drives human monocytes to CD16 positive macrophages. *J. Inflamm.* **46,** 78–85.
54. Yamamoto, T., Nagayama, K., Satomura, K., Honda, T., Okada, S. (2000) Increased serum IL-10 and endothelin levels in hemolytic uremic syndrome caused by *Escherichia coli* O157. *Nephron* **84,** 326–332.
55. Proulx, F., Litalien, C., Turgeon, J. P., Mariscalco, M. M., Seidman, E. (2000) Circulating levels of transforming growth factor-β1 and lymphokines among children with hemolytic uremic syndrome. *Am. J. Kidney Dis.* **35,** 29–34.
56. Scherberich, J. E. (2003) Proinflammatory blood monocytes: main effector and target cells in systemic and renal disease; background and therapeutic implications. *Int. J. Clin. Pharmacol. Ther.* **41,** 459–464.
57. Herra, C. M., Keane, C. T., Whelan, A. (1996) Increased expression of Fc γ receptors on neutrophils and monocytes may reflect ongoing bacterial infection. *J. Med. Microbiol.* **44,** 135–140.
58. Fingerle-Rowson, G., Angstwurm, M., Andreesen, R., Ziegler-Heitbrock, H. W. (1998) Selective depletion of CD14+ CD16+ monocytes by glucocorticoid therapy. *Clin. Exp. Immunol.* **112,** 501–506.
59. Kummerle-Deschner, J. B., Hoffmann, M. K., Niethammer, D., Dannecker, G. E. (1998) Pediatric rheumatology: autoimmune mechanisms and therapeutic strategies. *Immunol. Today* **19,** 250–253.