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Human CD16$^+$ and CD16$^-$ monocyte subsets display unique effector properties in inflammatory conditions in vivo

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ABSTRACT

Two major subsets of human Mo are identified based on CD14 and CD16 expression: the classical CD16$^-$ Mo and the minor CD14$^+$CD16$^+$ Mo. In vitro studies suggested distinct function and differentiation potential for each cell population. However, the in vivo relevance of these findings remains unclear. To evaluate the development and function of human Mo in an in vivo model, we transferred both Mo subpopulations into the peritoneum of immunocompromised mice in homeostatic or inflammatory conditions. Inflammation was induced with soluble LPS or particulate zymosan. CD16$^+$ were more phagocytic and produced higher amounts of TNF and IL-6 than CD16$^-$ Mo early after transfer with zymosan. They also produced higher levels of β2-defensin in any condition evaluated, which could represent a new marker for this subpopulation. In contrast, differentiating CD16$^-$ Mo (24 h after transfer) acquired greater APC capacity in LPS-induced peritonitis, whereas none of the Mo subsets attained this ability with zymosan. CX$_3$CL1 supported the survival of both Mo subsets in vivo. Similar Mo subpopulations were present in human peritonitis. These results support the idea of specialized roles of the Mo subset, where CD16$^+$ might act in an immediate innate immune response, whereas CD16$^-$ could have a major role as APCs. *J. Leukoc. Biol.* 90: 1119–1131; 2011.

Introduction

In humans, two major subsets of circulating Mo can be distinguished based on CD14 and CD16 expression. The classical CD16$^-$ Mo account for 85–90% of total Mo and have high levels of CD14 and no expression of CD16. The minor CD16$^+$ Mo subpopulation comprises the remaining 10–15% [1–4]. CD16$^+$ Mo can be subdivided further into cells with a high level of CD14 and low CD16 (intermediate CD14^{++}CD16$^+$ Mo) and cells with a low level of CD14 and high expression of CD16 (nonclassical CD14$^+$CD16^{++} Mo) [5]. Hierarchical clustering analysis of gene expression profiles showed that intermediate and nonclassical Mo are closely related, whereas classical and nonclassical Mo are more distantly clustered [6]. Phenotypically, CD16$^+$ are considered more mature cells than CD16$^-$ Mo [7] by their low expression of CD11b and CD33 and their high expression of CD16 and MHC class II molecules [8]. Human Mo subpopulations also differ in chemokine receptor expression. CD16$^-$ Mo have higher levels of inflammatory CCR1, CCR2, CXCR1, and CXCR2 receptors, as well as CCR7 and CD62L, two proteins involved in migration to lymphoid organs, whereas CD16$^+$ Mo have a higher expression of the CX$_3$CR1 receptor [9]. Binding of CX$_3$CR1 to its unique ligand CX$_3$CL1 promotes the survival of mouse Mo in vivo [10, 11] and of both subpopulations of human Mo in vitro [11]. Functional studies showed that CD16$^+$ Mo are the main source of TNF and IL-1 when stimulated with TLR2 and TLR4 ligands [3, 11, 12]. Together, with a reduced ability to produce IL-10, these data led to the conclusion that CD16$^+$ Mo are potent inducers of inflammation [13]. Moreover, the CD16$^+$/CD16$^-$ Mo ratio in blood significantly increased in chronic inflammatory conditions, such as sepsis [14], HIV infection [15], solid tumors [16], or rheumatoid arthritis [17]. Therefore, CD16$^+$ Mo were considered as "inflammatory" [7,

Abbreviations: 16–MDC=CD16$^-$ monocyte-derived cell, 16+MDC=CD16$^+$ monocyte-derived cell, CD16$^-$ Mo=CD14^{++}CD16$^-$ monocyte(s), FSC=forward-scatter, Mϕ=macrophage(s), MDC=monocyte-derived cell(s), MFI=mean fluorescence intensity, Mo=monocyte(s), sCX$_3$CL1=soluble CX$_3$CL1, SSC=side-scatter, Zym-FITC=zymosan coupled to FITC

The online version of this paper, found at www.jleukbio.org, includes supplemental information.

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18]. Regarding their potential for in vitro differentiation, $CD16^+$ Mo have increased capacity to differentiate into DCs and Mϕ, whereas $CD16^-$ Mo are prone to differentiate into Mϕ [19–21].

In mice, two major Mo subpopulations can also be identified. The $Ly6C^+$ subset resembles human $CD16^-$ Mo, and both express CCR1, CCR2, CXCR1, CXCR2, CCR7, and CD62L. Its counterpart, the $Ly6C^{lo}$ subset, shares some features with human $CD16^+$ Mo, such as their high expression of CX_3CR1 [2, 3, 22]. There is some controversy about the inflammatory properties of each cell population [2, 18, 23, 24]. However, recent studies suggest that $Ly6C^{lo}$ Mo are the first to be recruited into inflammatory sites, partially owed to their greater interaction with vascular endothelia [23]. Upon extravasation, they increase the expression of molecules associated with recruitment of new, inflammatory cells and with recognition and elimination of microorganisms; later, they initiate a differentiation program toward alternatively activated M2 Mϕ. In contrast, $Ly6C^+$ Mo arrive later at sites of inflammation and could differentiate into inflammatory M1 Mϕ or DCs [23]. The transcription profiles of the equivalent Mo subpopulations in human and mice are found to be similar [24].

Mo are plastic cells that modify their functional activity in response to environmental signals, which are difficult to reproduce in vitro. To directly investigate the role of Mo in vivo at the site of inflammation, we used an experimental condition in which the two human Mo subsets were transferred into the inflamed peritoneum of immunodeficient mice. Our results show that $CD16^-$ and $CD16^+$ Mo respond differently in terms of phagocytosis, cytokine production, adaptive immune system activation, and survival. The detection of $CD14^+CD16^-$ and $CD14^+CD16^+$ cells in human peritonitis leads us to suggest that these cells could participate in the regulation of inflammatory processes.

MATERIALS AND METHODS

Animals

The transfer of human Mo was performed into 2- to 4-month-old C.B-17 *scid-beige* mice (Taconic Farms, Germantown, NY, USA). *scid-beige* mice have a combined effect of *scid* (no T and B cells) and *beige* (defect in lysosomal trafficking regulator gene) mutations. These mice display, in addition to the T and B deficiency, a severely reduced NK cell function and phagocytosing cells [25]. Mice breeding, maintenance, and experimentation were conducted in accordance with the Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Mexico City, Mexico) institutional guidelines.

Mo subset isolation and transfer into mice

PBMCs were obtained by ficoll density gradient (Nycomed Pharma, New York, NY, USA) of buffy coats from healthy donors after signed informed consent. $CD16^-$ and $CD16^+$ Mo were purified by positive selection using magnetic separation systems (MACS, Miltenyi Biotec, Auburn, CA, USA), as reported previously [26]. $CD4^+$ T lymphocytes were purified by negative selection using a MACS isolation kit. Human $CD16^+$ and $CD16^-$ Mo ($3–5\times10^6$ cells) were transferred separately into the peritoneum of mice, previously treated with 200 μl LPS from *Escherichia coli* 0111:B4 (1 μg/mouse; Sigma-Aldrich, St. Louis, MO, USA) or 200 μl zymosan from *Saccharomyces cerevisiae* (100 μg/mouse; Molecular Probes, Eugene, OR, USA), 6 h or 1 h before the transfer of human Mo, respectively. Mice were also treated with 200 μl PBS as a control.

Detection of cytokines in mice

Peritoneal fluid from mice was obtained by washes with PBS, 1 h after PBS or zymosan injection or 6 h after LPS injection, to evaluate the cytokine milieu that human Mo will contact at the time of their transfer into the mouse peritoneum. Supernatant was separated by centrifugation. Detection of mouse IL-12, TNF, CCL2, IL-10, and IL-6 was performed by the CBA method (BD Biosciences, Bedford, MA, USA). Detection of mouse sCX_3CL1 was carried out with an ELISA kit (R&D Systems, Minneapolis, MN, USA).

Phagocytosis assay

Zym-FITC (100 μg; Molecular Probes) was injected into the peritoneal cavity of mice. One hour after stimulation, the Mo subpopulations were transferred into the mice; 1 h after transfer, mice were killed. Cells from the peritoneal cavity were obtained by lavage and stained with a mouse mAb against HLA-DR conjugated with PE (G46-6; BD Biosciences). This mAb does not cross-react with murine cells (not shown). Along the study, peritoneal cells were stained with this anti-HLA-DR mAb to differentiate human from mouse cells and gated according to the FSC/SSC profile of live Mo (except for the annexin V assays). The percentage of annexin V^- cells within this gate ranged 88–98% (Supplemental Fig. 1B). Data were acquired in a FACSCalibur (BD Biosciences) or a Cyan (Dako, Carpinteria, CA, USA) flow cytometer and analyzed using CellQuest software (BD Biosciences) or Summit 4.3 software (Dako).

Phenotypic characterization of Mo subpopulations

Subsets of human Mo were transferred into the peritoneum of mice as described. After 24, 48, or 72 h, peritoneal fluid was obtained by washes with PBS. Heparinized blood was obtained by cardiac puncture in anesthetized mice, as well as the spleen, lungs, and liver. The organs were sliced, incubated for 45 min at 37°C with collagenase D (1 mg/mL; Roche, Switzerland), and then disaggregated mechanically. Mononuclear cells from mouse blood and organs were obtained by ficoll gradient. These cells were stained with an anti-HLA-DR mAb conjugated to PE. The presence and phenotype of human cells in the mouse peritoneum were analyzed using mAb to human HLA-DR, CD14 (M5E2), CD16 (3G8), CD83 (HB15e), CD86 [2331 (FUN-1)], and CX_3CR1 (2A9-1). All mAb were from BD Biosciences except for CX_3CR1 (MBL, Japan). The same evaluation was also performed in freshly purified Mo. TLR expression was analyzed additionally in Mo using mAb to human TLR2 (TL2.1) and TLR4 (HTA125), from eBioscience (San Diego, CA, USA). Cells were analyzed by flow cytometry.

Production of intracellular cytokines and β_2-defensin

Human Mo subsets were transferred into mice as described. Three hours or 24 h later, cells were obtained from the peritoneal cavity of mice and cultured in supplemented RPMI-1640 medium for 3 h with brefeldin A (BD Biosciences). RPMI-1640 medium was supplemented with 10% heat-inactivated FBS, 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM nonessential amino acids (Hyclone Laboratories, Logan, UT, USA), 1% penicillin/streptomycin, and 50 μM 2-ME (Gibco BRL, Gaithersburg, MD, USA). After that, cells were stained with a mAb to HLA-DR. Then, cells were treated with Cytofix/Cytoperm solutions (BD Biosciences) and stained with anti-human TNF (MAb11), IL-6 (MQ2-13A5), or IL-12 (C8.6) mAb (BD Biosciences). Subsequently, polyclonal goat anti-mouse Igs [fraction $F(ab')_2$; Dako] for TNF or avidin (BD Biosciences) for IL-6 and IL-12 coupled to FITC were added. Detection of β_2-defensin was performed using a rabbit polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) and then a polyclonal goat anti-rabbit IgG coupled to Alexa Fluor 488 (Invitrogen, Carlsbad, CA, USA). IgGs from rabbit preimmune serum were used as a negative control of the anti-β2-defensin primary antibody.

Allogeneic proliferation assays and IFN-γ production by $CD4^+$ T lymphocytes

Purified $CD16^+$ and $CD16^-$ Mo from peripheral blood were used directly as APCs with $CD4^+$ T cells or transferred into the mouse peritoneum, treated previously with PBS, zymosan, or LPS, as described above. Human peritoneal cells were isolated 24 h later by FACS with an anti-human CD45 mAb coupled to FITC (HI30; BD Biosciences) in a MoFlo cell sorter (Dako). The subsets of freshly isolated Mo and the cells derived from them generated in vivo (MDC) were cocultured with CFSE-labeled (10 μM; Molecular Probes) human allogeneic $CD4^+$ T lymphocytes at a 1:10 Mo:T cell ratio for 5 days. Proliferation was determined by CFSE dilution. Occasionally, lymphocytes were harvested after 2 days of coculture, washed, and incubated in fresh medium for 6 h in the presence of brefeldin A. Subsequently, cells were treated with Cytofix/Cytoperm solutions and incubated with an anti-human IFN-γ mAb coupled to allophycocyanin (Miltenyi Biotec).

Evaluation of cell death

Three hours or 9 h after the transfer of Mo subsets into the peritoneum of mice, peritoneal cells were obtained and incubated with an anti-HLA-DR mAb coupled to PE. Then, cells were stained with biotin-coupled annexin V (BD Biosciences), which was detected with allophycocyanin-labeled streptavidin (BD Biosciences) in cells gated, as in Supplemental Fig. 1A. For in vitro assays, $CD16^-$ and $CD16^+$ Mo (1×10^6) were cultured for 6 h in supplemented RPMI-1640 medium without FBS to induce cell death by serum withdrawal in the presence or absence of mouse CX_3CL1 (100 ng; R&D Systems). Then, cells were stained using annexin V, as mentioned above. PI (BD Biosciences) was added to cells prior to analysis by flow cytometry.

In vivo CX_3CR1 blockade

Mice were treated with LPS as described previously. One hour before Mo transfer, 100 μg neutralizing rabbit anti-mouse CX_3CR1 polyclonal antibody (Torrey Pines Biolabs, San Marcos, CA, USA) was injected into the mouse peritoneal cavity. As a control, the same amount of antibody obtained from rabbit preimmune serum was used. After Mo transfer, a second dose of 100 μg neutralizing or control antibodies was injected. Cells from the peritoneal cavity were isolated 24 h later, stained with PE-labeled anti-HLA-DR mAb, and analyzed by flow cytometry.

Analysis of cells from the peritoneal cavity of patients with peritonitis

Cells from the peritoneal cavity of eight patients on peritoneal dialysis were obtained after their informed consent. The patients' condition was classified into inflammatory (four patients) or homeostatic (four patients), according to the presence or absence of neutrophils in the peritoneal lavage, respectively. Two of these patients were diagnosed with *Staphylococcus aureus* infection associated to inflammation, whereas the cause of inflammation in the remaining patients was not determined. A fraction of the peritoneal cells was incubated with mAb to CD14 labeled with PerCP (4G7; BD Biosciences), to HLA-DR coupled to PE, and to CD16 or CX_3CR3 labeled with FITC. Another fraction was used to purify $CD16^+$ and $CD16^-$ Mo by density gradient and the MACS system, as mentioned above for PBMC. The expression of CD16, CD14, HLA-DR, CX_3CR1, and β_2-defensin was evaluated in these cells by FACS analysis.

Statistical analysis

Data were expressed as mean ± SD of independent experiments. Statistical significance was determined by the paired Student's *t* test. Statistical significance was set at $P < 0.05$.

RESULTS

Cytokine milieu in the peritoneum of mice treated with LPS or zymosan

To evaluate the function of human $CD16^+$ and $CD16^-$ Mo under inflammatory conditions in vivo, we generated two inflammatory environments in the peritoneum of *scid-beige* mice by injecting zymosan or LPS. Mo were transferred into the peritoneal cavity of these mice 1 h after zymosan or 6 h after LPS. PBS was injected as a control. First, we analyzed the cytokine/chemokine expression profile in the mouse peritoneum before Mo transfer. The peritoneal fluid had low-to-undetectable amounts of IL-12, TNF, CCL2, and IL-6, but IL-10 was found at levels ~100 pg/ml. Inflammatory stimuli did not change IL-10 or IL-12 production. Zymosan induced higher production of IL-6 and TNF compared with LPS at the times evaluated, but LPS led to greater production of CCL2 (**Fig. 1**). These results indicate that LPS and zymosan induced different inflammatory milieus in the mouse peritoneum, which has constitutive expression of IL-10.

With regard to the species specificity of murine cytokines, mouse CCL2 has full activity on human cells [27], whereas murine IL-6 and IL-10 are only active on murine cells [28, 29]. TNF exerts variable degrees of species preference. Despite that there is a higher specific activity in the homologous system, murine and human TNFs behave similarly regarding in vitro cytolytic/cytostatic activity on human cell lines [30, 31].

Phenotype of human Mo subpopulations

Subsequently, to compare the potential changes induced in vivo, we evaluated the phenotype of freshly purified human $CD16^-$ and $CD16^+$ Mo before transfer into the mouse peritoneum. Our findings were similar to that reported previously

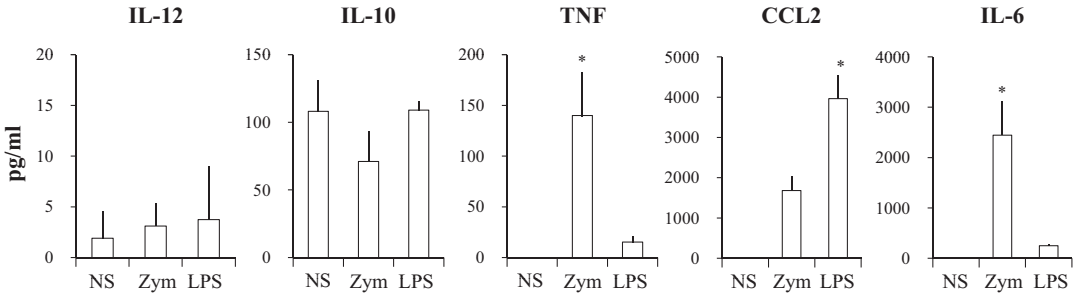

Figure 1. Inflammatory milieu generated in vivo by LPS and zymosan. Peritoneal fluid of mice was obtained by washes after stimulation with 1 μg LPS for 6 h, with 100 μg zymosan for 1 h (Zym), or with PBS for 1 h [no stimulus (NS)]. Mouse cytokines were measured by the CBA method. Results are expressed as mean ± SD of three independent experiments. Statistical analysis: $^*P < 0.05$.

[1, 2, 8]. $CD16^+$ Mo had higher expression of HLA-DR, CX_3CR1, and CD86 and variable expression of CD14. Both Mo subpopulations were negative for the expression of the mature DC-associated marker CD83. Previous studies have reported that human Mo produce the antimicrobial peptide β_2-defensin [32]; our analysis demonstrated that $CD16^+$ Mo have increased expression of this molecule compared with $CD16^-$ cells (**Fig. 2A**). No significant differences were found regarding TLR2 and TLR4 expression, two main receptors for zymosan and LPS, respectively (Fig. 2B).

$CD16^+$ Mo show increased effector capacity in response to zymosan particles

Once the human Mo subsets and the cytokine profile generated in the mouse peritoneum were characterized, we separately transferred $CD16^+$ and $CD16^-$ Mo populations into the peritoneal cavity of mice. To avoid massive cell death, Mo transfer was delayed 6 h after LPS injection (ref. [33], and data not shown). In contrast, Mo were transferred only 1 h after zymosan injection to prevent its removal by murine phagocytes.

Initially, we analyzed cytokine production of freshly purified Mo and of Mo 3 h after transfer into the mouse peritoneum. $CD16^+$ Mo exposed to zymosan were able to produce higher amounts of TNF and IL-6 than $CD16^-$ Mo at the single cell level (**Fig. 3A**). The production of these cytokines showed no significant differences between the Mo subsets with LPS and was almost undetectable in the absence of inflammation (Fig. 3A). The percentage of cells producing each cytokine did not show significant differences between the Mo subsets (data not shown). Moreover, the percentage of $CD16^+$ Mo producing β_2-defensin was augmented significantly with respect to $CD16^-$ Mo, regardless of the signals to which they were exposed, although $CD16^-$ Mo increased β2-defensin production in inflammation (Fig. 3B). Therefore, the expression of β_2-defensin could be considered as a novel marker to differentiate the two subpopulations of human Mo under homeostatic and inflammatory conditions.

Next, we evaluated the in vivo phagocytic ability of the human Mo subsets. Zym-FITC was injected into the mouse peritoneum, and 1 h later, the human Mo subpopulations were transferred. Peritoneal cells were obtained 1 h after transfer. The results showed that $CD16^+$ have greater capacity to internalize Zym-FITC than $CD16^-$ Mo in the percentage of phagocytic cells, as well as in the number of particles ingested per cell (Fig. 3C).

Altogether, our data suggest a major involvement of $CD16^+$ Mo in innate resistance to yeast cell derivatives, based on their phagocytosis ability and on their production of inflammatory mediators. In contrast, both Mo subsets had similar responses to LPS at early times.

Phenotypical changes of Mo subpopulations in vivo

Subsequently, we evaluated the phenotype of the Mo subsets at 24 h and 48 h after transfer into the mouse peritoneum to seek out whether they have preferential differentiation pathways toward DCs or Mϕ. The analysis was performed on HLA-DR^+ cells to identify human Mo, and therefore, these cells were considered as 100% of total. There was an increase in the levels of HLA-DR, CD83, and CD86 under inflammatory conditions with respect to their expression in freshly isolated Mo but also when Mo were transferred into the peritoneum in the absence of inflammation. The greatest increase of HLA-DR was induced with LPS, mainly in $CD16^-$ Mo. The augmentation of CD83 was transient, and the percentage of $CD83^+$ cells tended to diminish at 48 h post-transfer compared with the cells evaluated at 24 h. As for the expression of CD14 and CD16, two proteins that define the Mo subpopulations, we found that $CD16^-$ and $CD16^+$ Mo tended to maintain the expression of CD14, although $CD16^+$ Mo significantly increased

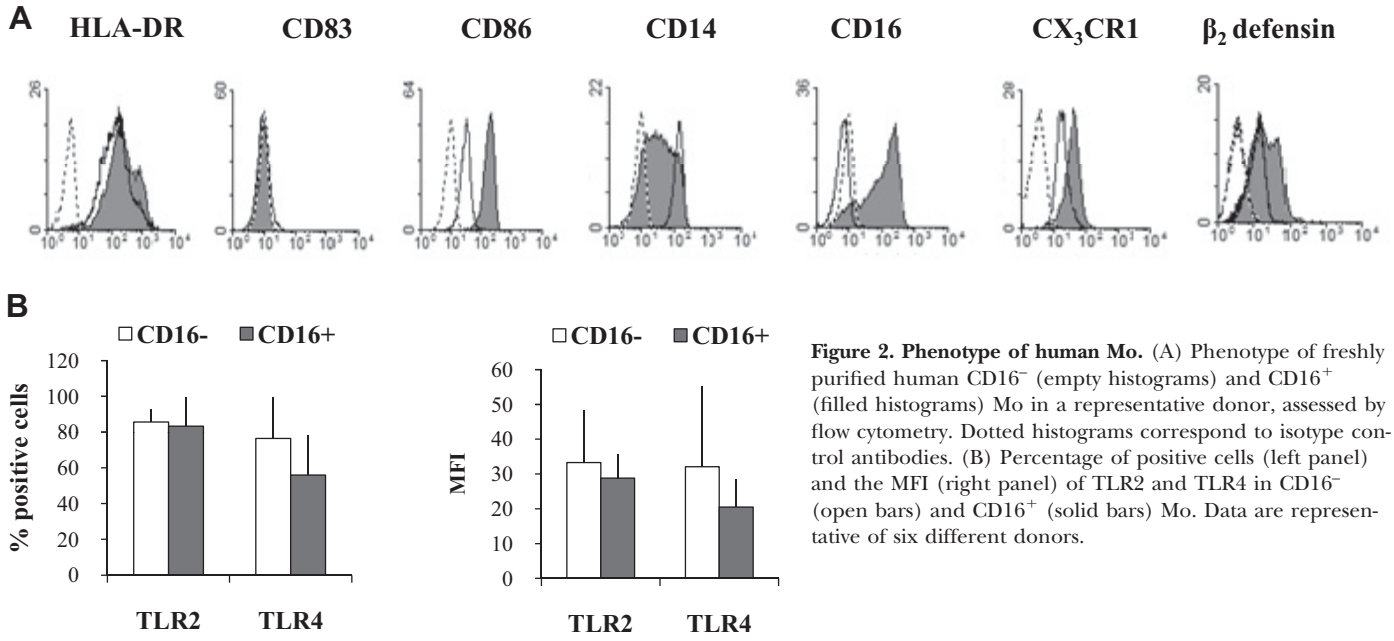

Figure 2. Phenotype of human Mo. (A) Phenotype of freshly purified human $CD16^-$ (empty histograms) and $CD16^+$ (filled histograms) Mo in a representative donor, assessed by flow cytometry. Dotted histograms correspond to isotype control antibodies. (B) Percentage of positive cells (left panel) and the MFI (right panel) of TLR2 and TLR4 in $CD16^-$ (open bars) and $CD16^+$ (solid bars) Mo. Data are representative of six different donors.

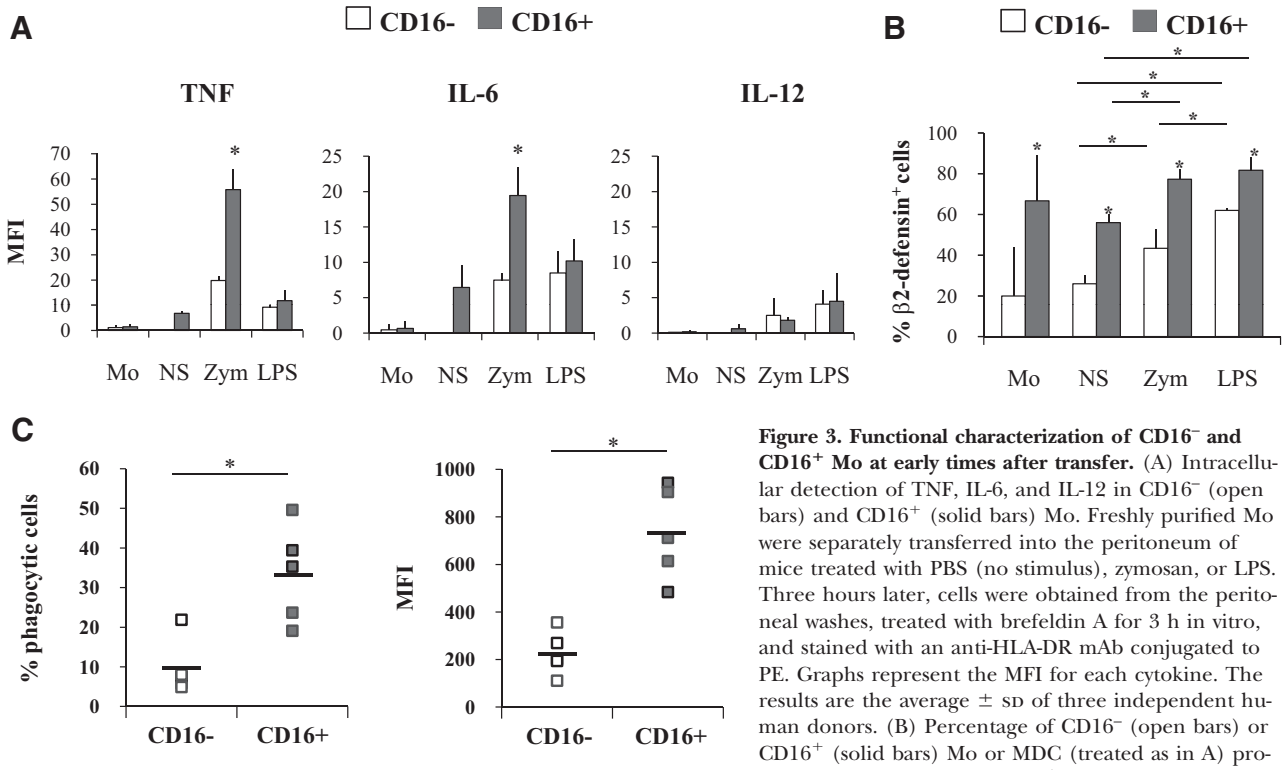

Figure 3. Functional characterization of CD16$^-$ and CD16$^+$ Mo at early times after transfer. (A) Intracellular detection of TNF, IL-6, and IL-12 in CD16$^-$ (open bars) and CD16$^+$ (solid bars) Mo. Freshly purified Mo were separately transferred into the peritoneum of mice treated with PBS (no stimulus), zymosan, or LPS. Three hours later, cells were obtained from the peritoneal washes, treated with brefeldin A for 3 h in vitro, and stained with an anti-HLA-DR mAb conjugated to PE. Graphs represent the MFI for each cytokine. The results are the average ± SD of three independent human donors. (B) Percentage of CD16$^-$ (open bars) or CD16$^+$ (solid bars) Mo or MDC (treated as in A) producing β_2-defensin. Graphs represent the mean ± SD of three separate donors. (C) Purified CD16$^-$ and CD16$^+$ Mo from five independent human donors were transferred into mice treated with Zym-FITC. Peritoneal cells were obtained 1 h later and stained with a mAb to HLA-DR labeled with PE. Shown is the percentage of cells that internalized zymosan (left) and the MFI of phagocytic cells (right) in CD16$^-$ (open squares) and CD16$^+$ (solid squares) cell subpopulations. Each square represents a different donor, and horizontal bars depict the mean for each set of values. Peritoneal cells were gated on HLA-DR$^+$ human cells (100%). Statistical analysis: $^*P < 0.05$.

the percentage of CD14$^+$ cells with LPS. The three conditions led to the expression of CD16 on CD16$^-$ Mo, whereas some CD16$^+$ cells lost the expression of this marker (**Fig. 4A** and **B**).

As mentioned above, changes in Mo phenotype were evident in the absence of inflammation. To evaluate whether the only presence of human Mo caused inflammation in the mouse peritoneum, we analyzed the cytokine profile of the peritoneal fluid under no stimulation compared with LPS- and zymosan-induced peritonitis. Thus, mice were injected with PBS, zymosan, or LPS, and 1 h (PBS and zymosan) or 6 h (LPS) later, CD16$^-$ and CD16$^+$ Mo were transferred. Then, 3–6 h after Mo transfer, peritoneal washes were obtained, and the amount of TNF, IL-6, CCL2, and IL-12 was evaluated. The times for collecting the peritoneal fluid were chosen based on the number of murine-infiltrating cells, which peaked at 4 h after the injection of zymosan or PBS and at 12 h after LPS (Supplemental Fig. 2A). Our data indicate that the sole presence of human Mo induced detectable levels of murine TNF, IL-6, and CCL2 (Supplemental Fig. 2B), suggesting that they evoked a certain degree of inflammation, although to a much lesser extent than zymosan or LPS.

In conclusion, both human Mo subsets change their phenotype in vivo, independently of the environment to which they are exposed. Their increase of HLA-DR, CD86, and CD83 expression could account for a preferential differentiation toward the DC pathway, although they preserve the expression of CD14.

CD16$^-$ Mo acquire greater capacity to activate CD4$^+$ T lymphocytes with LPS

Next, we analyzed whether the phenotype acquired by developing Mo correlated with more-efficient APC ability, as DCs are distinguished by their capacity to stimulate naive T cells. 16+MDC and 16–MDC were recovered by FACS after 24 h in the peritoneal cavity of mice and cocultured with allogeneic CD4$^+$ T lymphocytes. Freshly isolated Mo were also used as APCs for comparison. 16–MDC, recovered from LPS-treated mice, increased an average of three times its capacity to induce lymphoproliferation (**Fig. 5A**). In contrast, PBS or zymosan did not induce noticeable changes in 16–MDC APC ability. 16+MDC cells did not significantly alter their lymphoproliferative capacity in inflammation, with respect to their activity as freshly isolated Mo (Fig. 5A).

MDC increased the percentage of cells producing TNF and IL-6 after 24 h in the inflamed peritoneum of mice compared with controls. Interestingly, a higher percentage of 16+MDC produced TNF with respect to 16–MDC when exposed to zymosan. On the other hand, the percentage of IL-12-producing 16–MDC was elevated compared with 16+MDC in the presence of LPS (Fig. 5B). The increased production of IL-12 by

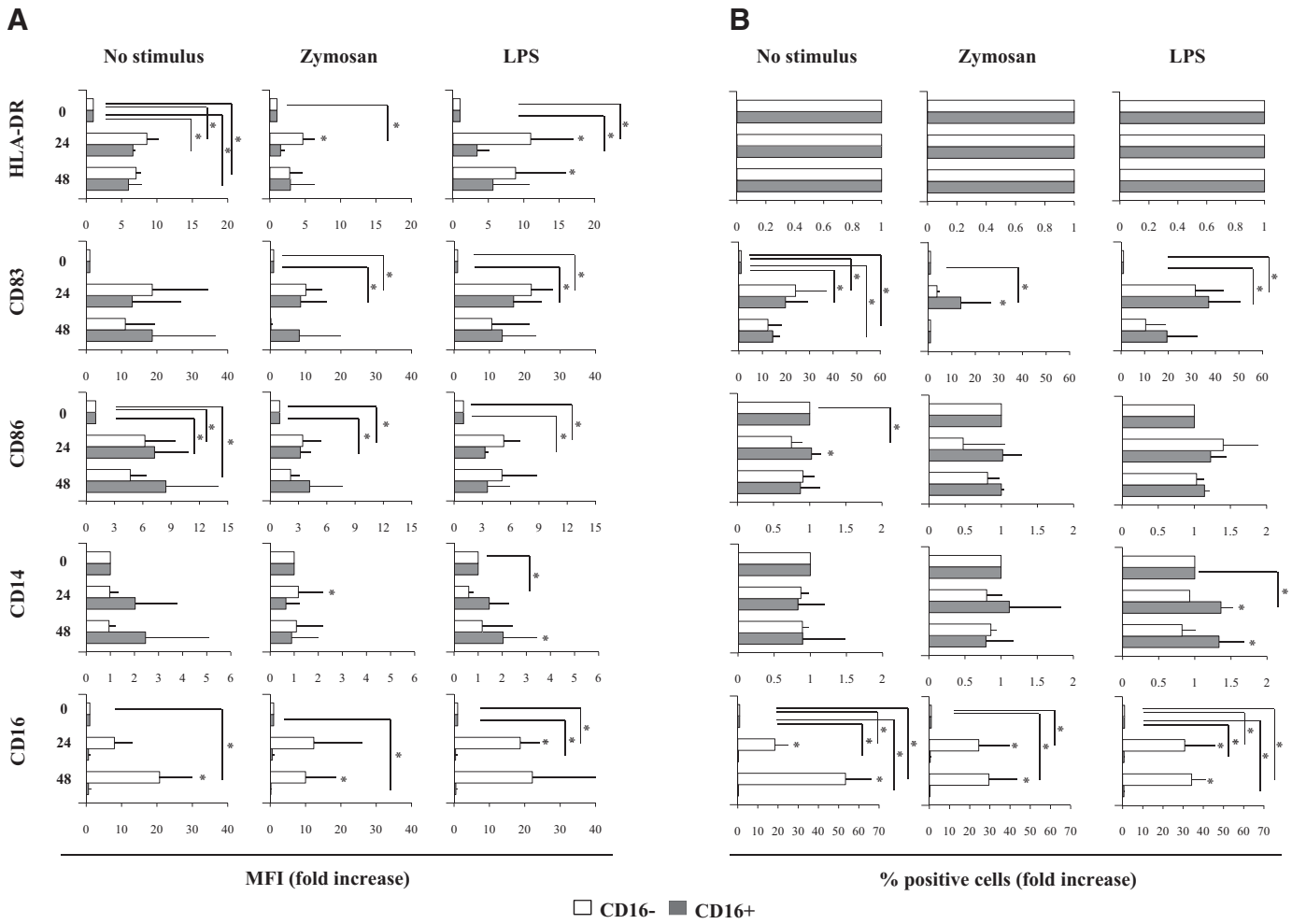

Figure 4. Phenotype of transferred human Mo subpopulations obtained from mouse peritoneal fluid. Freshly purified (0) $CD16^-$ (open bars) and $CD16^+$ (solid bars) Mo were separately transferred into the peritoneum of mice treated with PBS (No stimulus), zymosan, or LPS. Cells were obtained from the peritoneum 24 h or 48 h later. Then, cells were incubated with a mAb to HLA-DR labeled with PE and to CD83, CD86, CD14, and CD16 (all labeled with FITC). The bars represent the fold increase of the MFI (A) and the percentage of positive cells (B) for each marker, with respect to the values of Mo within the $HLA\text{-}DR^+$ gate. Relative numbers in Mo were set as 1. The results are the average of three human donors per treatment data point (three donors for the condition of no stimulus, three different donors for zymosan, and three separate donors for LPS). Statistical analysis: $^*P < 0.05$.

16–MDC led us to evaluate the induction of IFN-γ in allogeneic $CD4^+$ T lymphocytes stimulated by MDC. 16–MDC recovered from the inflammatory condition, induced by LPS were also major inducers of IFN-γ in noncycling and cycling cells (Fig. 5C). Moreover, even the two MDC subsets did not differ in IL-12 production with zymosan, developing 16–MDC slightly increased the numbers of cycling $CD4^+$ T cells that produce IFN-γ. In conclusion, 16–MDC reached greater ability to activate the adaptive immune system than 16+MDC under inflammatory environments in vivo.

Mo subpopulations have a different survival rate in the mouse peritoneum

Zymosan and LPS can induce leukocyte cell death through the release of several factors, including reactive nitrogen species and ROS, TNF, or IL-6 [33, 34]. Thus, we next evaluated the influence of LPS- and zymosan-induced peritonitis on human Mo survival. We obtained higher recovery of 16+MDC cells in inflammatory conditions induced by LPS at 24 h, whereas 16–MDC survived better in the presence of zymosan (**Fig. 6A** and **B**). The Mo recovery was low in any circumstance but consistent with other studies about grafted Mo [2]. Noteworthy, the absence of stimuli in the peritoneum led to a pronounced Mo death, and almost no human cells were recovered in this condition (Fig. 6C). Although we have proved that the sole presence of human Mo in the mouse peritoneum caused some degree of inflammation, their acute cell death suggests the lack of survival factors in this condition.

To explore whether phagocytosis of zymosan differentially affects the survival of human Mo, these cells were transferred into mice previously treated with zymosan; 3 h or 9 h later, cells were collected, and phagocytosis and cell death were ana-

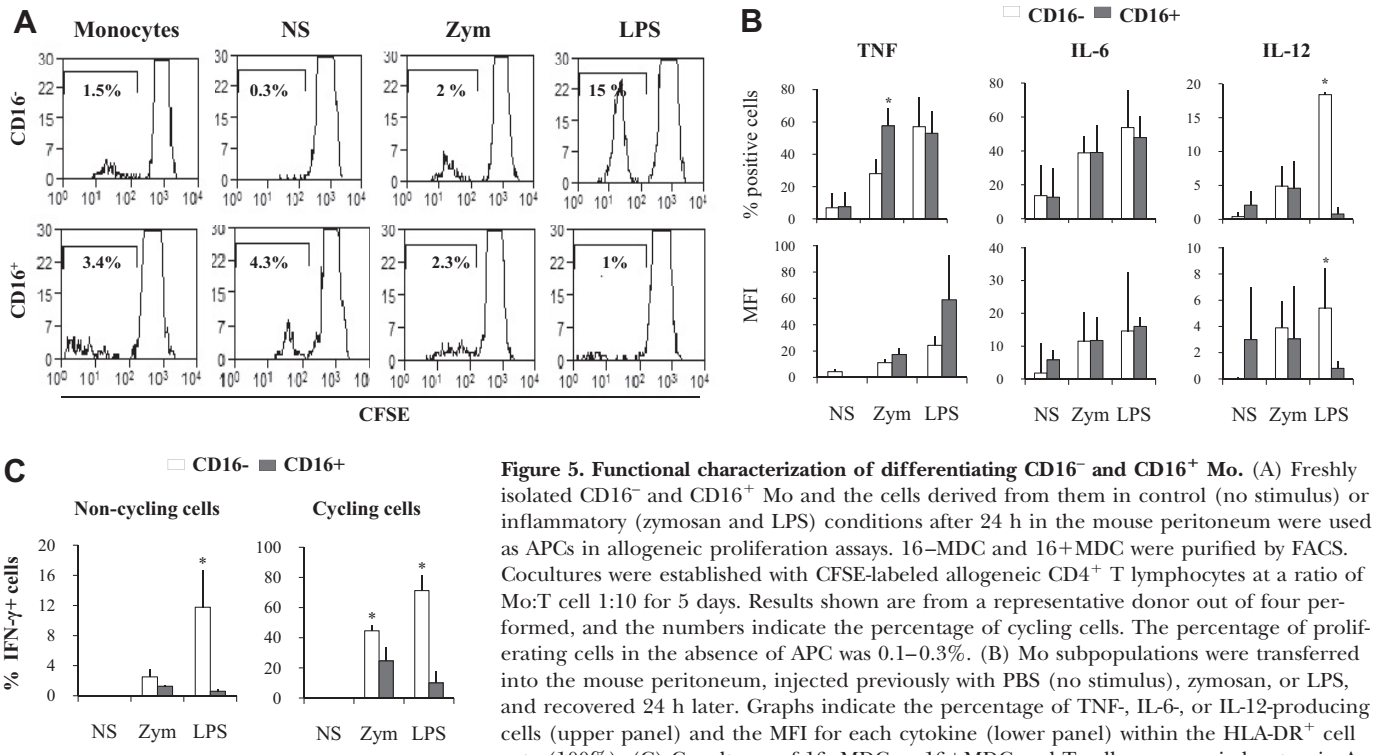

Figure 5. Functional characterization of differentiating CD16$^-$ and CD16$^+$ Mo. (A) Freshly isolated CD16$^-$ and CD16$^+$ Mo and the cells derived from them in control (no stimulus) or inflammatory (zymosan and LPS) conditions after 24 h in the mouse peritoneum were used as APCs in allogeneic proliferation assays. 16–MDC and 16+MDC were purified by FACS. Cocultures were established with CFSE-labeled allogeneic CD4$^+$ T lymphocytes at a ratio of Mo:T cell 1:10 for 5 days. Results shown are from a representative donor out of four performed, and the numbers indicate the percentage of cycling cells. The percentage of proliferating cells in the absence of APC was 0.1–0.3%. (B) Mo subpopulations were transferred into the mouse peritoneum, injected previously with PBS (no stimulus), zymosan, or LPS, and recovered 24 h later. Graphs indicate the percentage of TNF-, IL-6-, or IL-12-producing cells (upper panel) and the MFI for each cytokine (lower panel) within the HLA-DR$^+$ cell gate (100%). (C) Cocultures of 16–MDC or 16+MDC and T cells were carried out as in A for 2 days. Shown is the percentage of IFN-γ-producing CD4$^+$ T lymphocytes within the proliferating and nonproliferating cell populations. Each cell population was considered as 100% for data normalization. (B and C) The results are the average of three different human donors. Statistical analysis: $*P < 0.05$.

lyzed. At 3 h and 9 h, CD16$^+$ Mo had more phagocytic ability than CD16$^-$ cells (Supplemental Fig. 3A). However, zymosan uptake did not induce the death of a particular Mo subset (Supplemental Fig. 3B). Therefore, mechanisms other than phagocytosis might be involved in cell death. It is known that zymosan increased the expression of adhesion molecules [35] and that CD16$^+$ have higher levels of molecules related with adherence to endothelia (LFA-1, VLA-4, ICAM-1, CX_3CR1, among others) than do CD16$^-$ Mo [2, 8, 9]. An additional option to explain the poor recovery of CD16$^+$ Mo is that zymosan might selectively up-regulate in these cells the expression of molecules that mediate firm adhesive contacts with the surrounding mesothelial cells, avoiding their full recovery. To evaluate this possibility, we isolated the peritoneal membrane of mice treated with zymosan, 3 h post-transfer of human Mo. We found comparable numbers of CD16$^+$ and CD16$^-$ Mo in tight contact with mesothelial cells (Supplemental Fig. 4), which suggests that differential Mo adherence would not explain their recovery. However, this also indicates that we might be underestimating the number of human Mo that remained alive within the mouse peritoneum.

To determine whether the differences in Mo recovery were associated with migration into other tissues, mouse blood, spleen, lungs, and liver were analyzed for the presence of HLA-DR$^+$ cells. The data showed that both subpopulations of Mo can migrate into these tissues with no significant differences (Supplemental Fig. 5); in fact, the Mo subset with higher representation in the peritoneum was also more abundant in the tissues. Thus, the differences of Mo recovery in the peritoneum were not a result of migration but rather, to an in situ process. In agreement with this suggestion, the lower recovery of a given Mo subset in the peritoneum correlated with higher annexin V staining (Fig. 6D). Hence, the different rate of Mo death in situ could mirror the amount of cytotoxic, as well as survival, factors generated in the distinct environments.

The interaction CX_3CR1/CX_3CL1 is important for the survival of human Mo subsets

It is known that the interaction of CX_3CR1 with its ligand promotes the survival of human Mo subpopulations in vitro [11]. Thus, we explored whether CX_3CL1 was produced in the mouse peritoneum upon LPS or zymosan stimulation and whether the mouse chemokine also favored human Mo survival. The mouse peritoneum has undetectable levels of sCX_3CL1 in the absence of inflammation, and LPS and zymosan induced its production (**Fig. 7A**). CX_3CL1 production did not increase with the sole presence of Mo but decreased over time when CD16$^-$ or CD16$^+$ Mo were transferred into the mouse peritoneum in the presence of zymosan; the decline was faster with CD16$^+$ cells (Fig. 7A). Moreover, sCX_3CL1 tended to increase when CD16$^+$ Mo were transferred with LPS, whereas the behavior of CD16$^-$ cells was analogous to what was found with zymosan (Fig. 7A). These data imply that human Mo can modify sCX_3CL1 production in inflammatory sites or have different chemokine consumption. Subsequently, we observed that mouse sCX_3CL1 prevented cell death of cul-

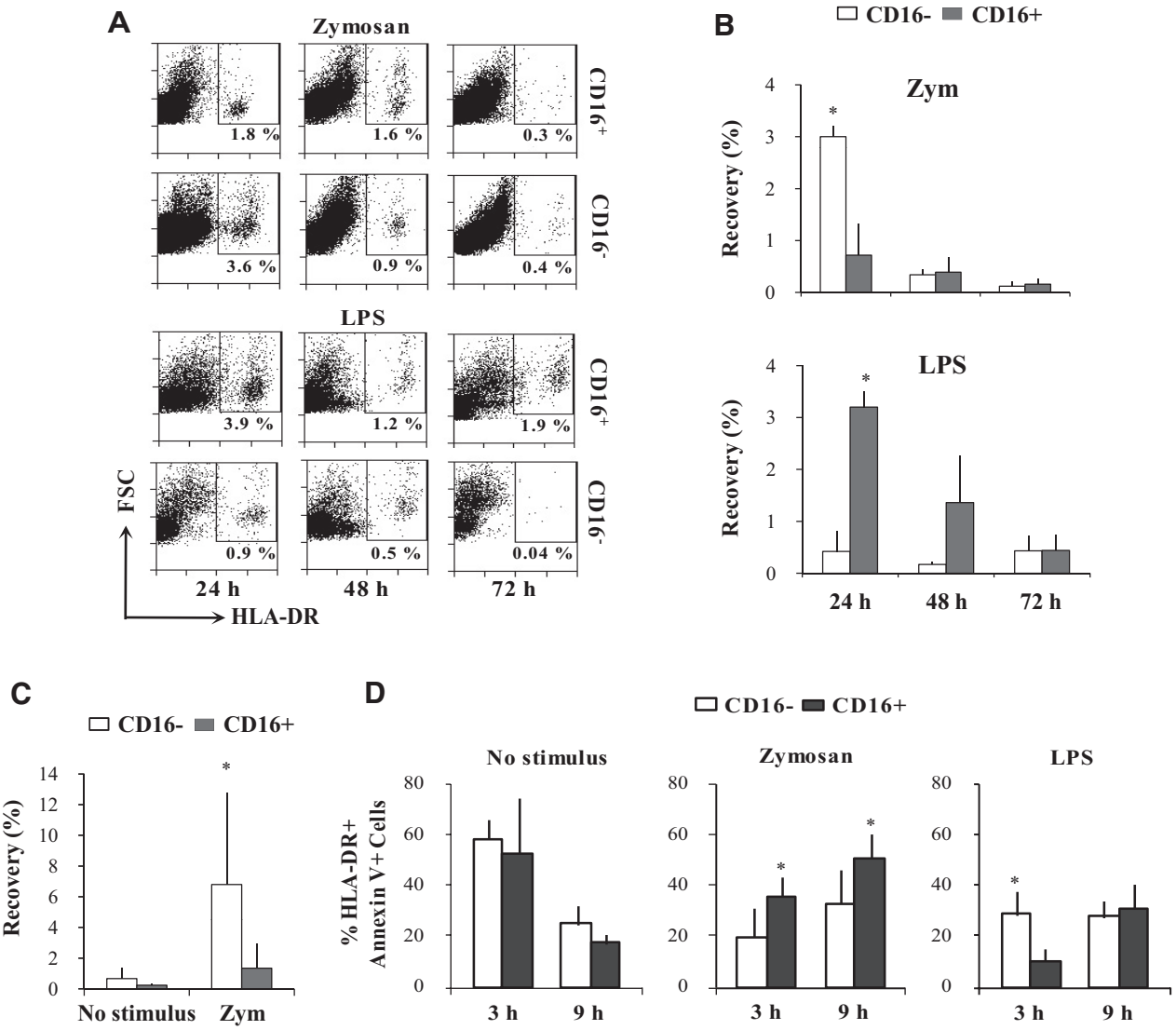

Figure 6. Recovery of Mo subpopulations in the mouse peritoneal cavity. $CD16^-$ and $CD16^+$ Mo were transferred into the peritoneal cavity of mice under homeostatic or inflammatory conditions. After 24, 48, and 72 h, peritoneal cells were obtained, counted, and stained with a mAb to HLA-DR labeled with PE. (A) Dot plots showing the percentage of $HLA\text{-}DR^+$ human cells in the mouse peritoneal cavity in a representative experiment. Mice were treated previously with zymosan (upper panels) or LPS (lower panels). Percentage of $HLA\text{-}DR^+$ cells gated in the marked region is indicated. (B) Recovery of human Mo subpopulations from the mouse peritoneum treated with zymosan (upper) or LPS (lower) at different times after transfer. Recovery was calculated as the percentage of human Mo obtained from the peritoneum with respect to the number transferred initially. Mo from the same three donors were transferred into LPS- and zymosan-treated mice. (C) $CD16^-$ and $CD16^+$ Mo from three separate donors were transferred into the peritoneum of mice treated previously with PBS (No stimulus) or with zymosan. The graph represents the recovery of Mo subsets 24 h later in each condition, calculated as in B. (D) Cells from the peritoneal cavity of mice treated with PBS (No stimulus), LPS, or zymosan were obtained 3 h or 9 h after Mo transfer. Then, they were stained with an anti-HLA-DR mAb and annexin V. Graphs represent the percentage of annexin V^+ cells within the $HLA\text{-}DR^+$ cell gate (100%) in $CD16^-$ (open bars) and $CD16^+$ (solid bars) Mo. The results are the average of three independent donors. Statistical analysis: $^*P < 0.05$.

tured human Mo subsets induced by serum deprivation (Fig. 7B). In agreement with previous reports [2], freshly isolated $CD16^+$ Mo had higher levels of CX_3CR1 than $CD16^-$ Mo (Figs. 2A and 7C). When both cell subpopulations were transferred into mice, $CD16^-$ Mo increased their CX_3CR1 expression in all conditions, whereas $CD16^+$ Mo showed nearly no significant changes (Fig. 7C).

To determine whether the interaction CX_3CR1/CX_3CL1 in vivo was significant for Mo survival, Mo subsets were injected into the inflamed peritoneum of mice treated with LPS, together with neutralizing antibodies to mouse CX_3CL1. Blockade of CX_3CL1 diminished the recovery of $CD16^+$ and $CD16^-$ Mo 24 h later (Fig. 7D and E). These results indicate that the interaction CX_3CR1/CX_3CL1 in vivo is relevant for the survival of Mo subsets; however, CX_3CR1 activation might not be responsible for the different maintenance of $CD16^+$ and $CD16^-$ Mo in inflammatory conditions.

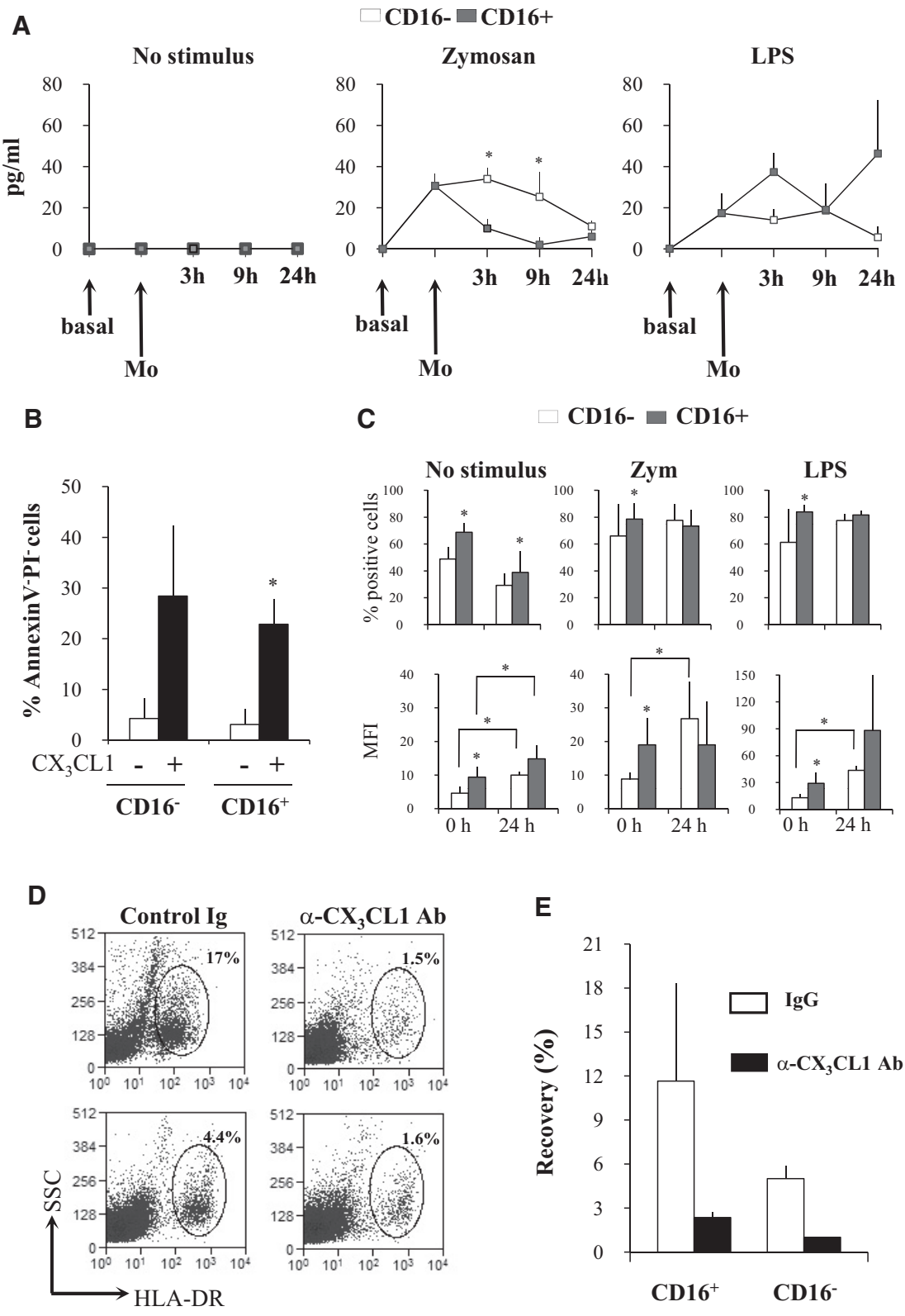

Figure 7. Mouse sCX_3CL1 is produced under LPS and zymosan stimulus and is important for human Mo survival. (A) sCX_3CL1 was measured in the peritoneal fluid of unstimulated mice (basal) and of mice treated for 1 h with PBS (No stimulus) or zymosan or for 6 h with LPS (Mo). At these times, $CD16^-$ and $CD16^+$ Mo were transferred into the mouse peritoneum; peritoneal fluid was obtained 3, 9, or 24 h later, and sCX_3CL1 levels were evaluated. (B) Freshly isolated Mo subsets were cultured in vitro in supplemented RPMI-1640 medium in the absence of serum and in the absence (open bars) or presence (solid bars) of mouse $rsCX_3CL1$. After 6 h, cells were stained with annexin V and PI, and cellular death was assessed by flow cytometry. Graph represents the percentage of annexin V^-PI^- Mo. (C) Evaluation of CX_3CR1 expression in both subpopulations of freshly purified Mo (0 h) and after 24 h in the mouse peritoneal cavity in the absence of stimulation (No stimulus) or in inflammatory conditions induced by LPS or zymosan. The results are the percentage of positive cells (upper panel) and MFI (lower panel) of CX_3CR1 within the $HLA\text{-}DR^+$ cell region (100%). (D) Mice were stimulated with LPS in the peritoneal cavity. One hour before and 1 h after the transfer of human Mo subsets, neutralizing antibodies to murine CX_3CL1 or irrelevant rabbit Igs (Control Ig) were injected. Peritoneal cells were obtained 24 h later, stained with a mAb to HLA-DR, and analyzed by flow cytometry. Numbers in the dot plots indicate the percentage of human cells ($HLA\text{-}DR^+$) within the total peritoneal cells. (E) Recovery of Mo subsets of the experiments in D, analyzed within the $HLA\text{-}DR^+$ cell gate, calculated as in Fig. 5B. (A–C) Results are the average of three independent experiments and of two in D and E. Statistical analysis: $^*P < 0.05$.

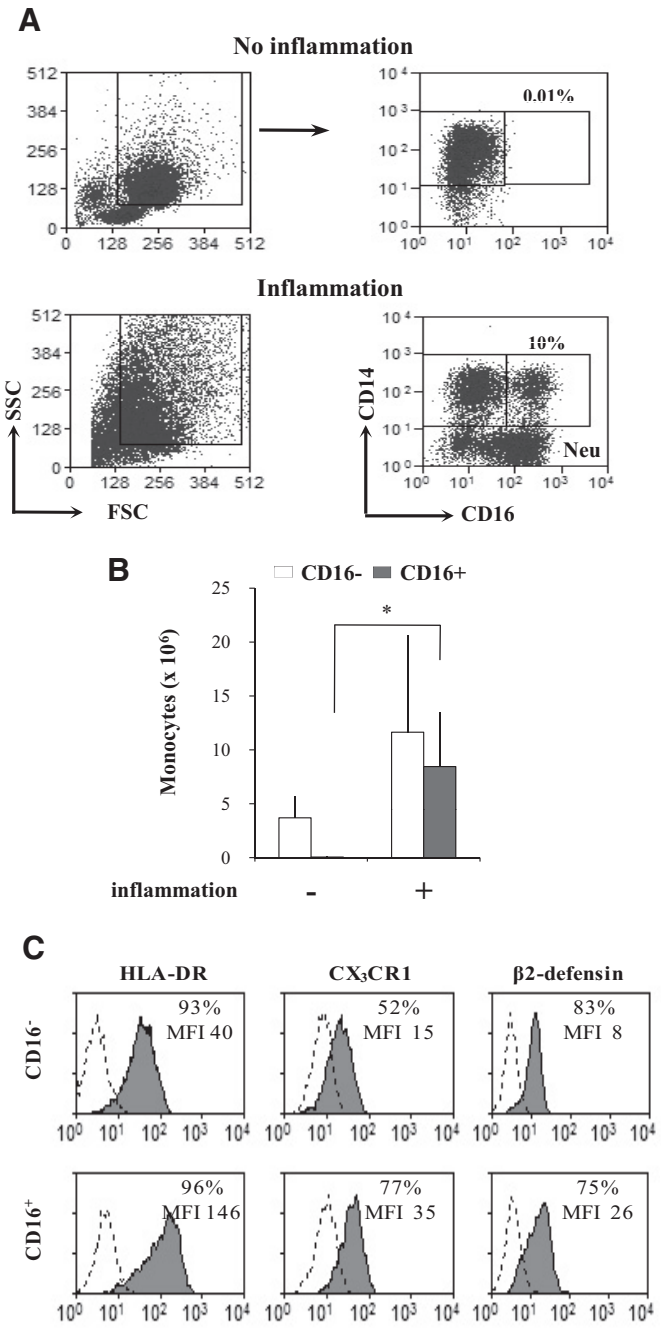

Figure 8. Phenotype and function of monocytic cells in human peritonitis. Cells from the peritoneal cavity of patients on peritoneal dialysis were stained with a mAb to CD14 labeled with PerCP and to CD16 labeled with FITC. (A) FSC and SSC profiles of peritoneal cells from patients without (upper) or with (lower) inflammation, showing the cells of greater size and complexity than lymphocytes gated for analysis (left) and the expression of CD14 and CD16 within the selected gate (right). Numbers represent the percentage of $CD14^+CD16^+$ cells in each condition; Neu, $CD14^-CD16^+$ neutrophils. (B) Amount of $CD14^+CD16^-$ (open bars) and $CD14^+CD16^+$ (solid bars) cells recovered from the peritoneal fluid of patients with (+) or without (–) inflammation. (C) HLA-DR, CX_3CR1, and β_2-defensin expression on $CD14^+CD16^-$ and $CD14^+CD16^+$ cells purified from the peritoneal fluid of patients with inflammation. Each molecule is represented with solid lines, and dotted lines show the profile of irrelevant control antibodies. The results are the average of four different donors per condition. Statistical analysis: $^*P < 0.05$.

$CD16^+$ and $CD16^-$ Mo subpopulations are found in the inflamed peritoneum of patients

To evaluate whether the Mo subpopulations were present in the human peritoneal cavity under inflammatory conditions, we analyzed the peritoneal fluid of patients on dialysis. We define homeostasic or inflammatory conditions by the absence or presence of $CD16^+HLA\text{-}DR^-$ neutrophils in the peritoneal fluid, respectively. In the absence of inflammation, there was an average of 3.7×10^6 $CD14^+CD16^-$ Mo per patient and very few $CD14^+CD16^+$ cells (**Fig. 8A**). Under inflammatory conditions, there was an increase in the number of $CD14^+CD16^-$ cells (11×10^6), which could be the result of blood $CD16^-$ Mo infiltration (Fig. 8B). In these individuals, we also found an infiltration of $CD14^+CD16^+$ cells (8.4×10^6), and these cells have higher expression levels of HLA-DR, CX_3CR1, and β_2-defensin than $CD14^+CD16^-$ cells (Fig. 8C).

DISCUSSION

Two main human Mo subsets have been described, primarily based on their CD16 expression, which might have specialized roles [1, 8, 12, 13]. $CD16^+$ cells are considered as inflammatory and more mature cells than $CD16^-$ Mo. $CD16^+$ Mo secrete high amounts of TNF in vitro [12, 15, 36] and low levels of IL-10 [13]. Moreover, they accumulate in the marginal pool, which may facilitate their rapid extravasation into inflammatory sites [37]. To investigate the function of the human Mo subsets in localized inflammatory sites, we generated two models of mouse peritonitis after LPS or zymosan injection, transferred the human $CD16^-$ and $CD16^+$ Mo into the peritoneal cavity, and assessed their phenotype, function, and survival rate. We used a reliable model to evaluate human cell differentiation, the immunocompromised *scid-beige* mice. These mice lack T and B cells and have a defect on lysosomal biogenesis, which results in defective NK function and low antibody-dependent cell cytotoxicity. These characteristics make these mice a model to theoretically improve the survival of xenogeneic cells purified by antibodies [38].

Our results showed that $CD16^+$ had greater ability to uptake zymosan particles than $CD16^-$ Mo. Their higher phagocytic capacity is different from that reported in vitro [26], where it was found that $CD16^-$ Mo internalized zymosan more efficiently. Another study showed that both Mo subpopulations phagocytosed germinated *Aspergillus fumigatus* conidia with equal efficiency in vitro; these fungi express β-glucans on their surfaces, similar to zymosan [36]. The molecular bases of these discrepancies are currently unknown. However, our results are in accordance with the reported data about preferential engulfment of latex beads by murine $Ly6C^{lo}$ Mo (homologous to human $CD16^+$ Mo) compared with $Ly6C^+$ Mo (homologous to human $CD16^-$ Mo) [39]. The presence of functional serum opsonins and other molecules of the inflammatory milieu,

which are absent in the in vitro assays, could modify the ability of Mo subsets to ingest zymosan. Thus, a well-characterized chemokine, CCL2, reduced the phagocytic ability of peritoneal Mϕ in vivo [40]. This chemokine is present under zymosan-induced inflammation. As only CD16$^-$ Mo express CCR2, the high-affinity CCL2 chemokine receptor, it is plausible that their phagocytic ability may be specifically down-regulated. Furthermore, inflammatory factors induced by zymosan could alter the expression of its receptors in vivo. However, preliminary results from our laboratory showed that the expression of CD11b and dectin-1, two major receptors of zymosan, is very similar between CD16$^+$ and CD16$^-$ Mo, transferred for 3 h into zymosan-treated mice (data not shown).

In addition to their greater phagocytic ability, CD16$^+$ Mo produce large amounts of TNF and IL-6 with zymosan at short times after transfer and of TNF at later times, suggesting that this Mo subset might have a specialized role against particulate antigens in vivo. These preferential responses to zymosan were not owed to higher TLR2 expression by CD16$^+$ Mo. In addition, CD16$^+$ produce greater amounts of β_2-defensin than CD16$^-$ Mo. Therefore, CD16$^+$ Mo could have more efficient innate responses against some microorganisms upon recruitment into inflammatory sites compared with CD16$^-$ cells. This idea is supported by microarray studies, where CD16$^+$ Mo had increased expression of genes associated with microbicidal activity (TNF, α-defensins, opsonins, lysosomal proteases), whereas CD16$^-$ Mo were associated with apoptotic cell removal and return to homeostasis [18]. However, another study showed contrasting results, derived from the up-regulation of genes involved in antimicrobial function in CD16$^-$ Mo (lysozyme, MPO, cathepsin G) [41]. Thus, our data might contribute to shed light on these discrepancies.

After 24 h in the mouse peritoneum, CD16$^-$ and CD16$^+$ Mo increased the expression of proteins related with their APC ability (HLA-DR, CD86), mainly under the inflammatory condition induced by LPS, and produced inflammatory cytokines. However, both Mo subsets maintained CD14 expression. In the condition of "no stimulus", the increase of HLA-DR, CD83, and CD86 expression was also observed; however, these Mo did not show capacity to produce inflammatory cytokines. Furthermore, 16–MDC became a more potent APC than 16+MDC in the presence of LPS. They induced greater proliferation and IFN-γ production in allogeneic CD4$^+$ T cells, which might be partially related to their increased production of IL-12. Similarly, we reported previously that DCs derived in vitro from CD16$^-$ Mo produced higher levels of IL-12 than CD16$^+$ Mo-derived DCs [42]. These data suggested that the inflammatory milieu induced by LPS, which contains high levels of CCL2, may drive CD16$^-$ Mo to acquire DC characteristics. The increased APC ability of 16–MDC in LPS-induced peritonitis might reside partially in their expression of CCR2, as CCL2 enhances T cell activation through up-regulation of MHC class II and costimulatory molecules in APCs [43], and 16–MDC showed increased expression of HLA-DR compared with 16+MDC. Despite the phenotypical changes, the poor APC ability developed by the Mo subsets in the absence of stimulation and in the presence of zymosan could be more related to the Mϕ differentiation pathway. Additionally, the absence of inflammatory cytokine production by MDC in the condition of no stimulus and the reported immunological tolerance induced by zymosan in DCs and Mϕ [44] may account for their inefficient APC ability. Overall, these data suggest that CD16$^+$ and CD16$^-$ Mo might differentiate in vivo into Mϕ or DCs, as it has been reported for their murine counterparts [10, 45–47], depending on the environmental conditions to which they are exposed.

Interestingly, CD16$^-$ Mo acquire CD16 expression in vivo, which would make it difficult to distinguish whether CD16$^+$ Mo in the inflammatory infiltrates derive from CD16$^-$ cells that have acquired CD16 or from circulating CD16$^+$ Mo. The expression of CD16 by CD16$^-$ Mo has been confirmed in vitro [8, 19] but not in vivo, and it is possible that cytokines responsible for CD16 induction, such as TGF-β1 [19], might be present in the mouse peritoneum. Some microarray studies suggest that the human Mo subsets have a common origin [21] and that intermediate Mo are the direct link between classical and nonclassical Mo [5, 6]. Furthermore, mouse Ly6C$^+$ Mo are able to differentiate into Ly6C^{lo} cells in the blood [48], similar to what might occur with the homologous cell populations in humans. In the peritoneal fluid of patients with peritonitis, we identified CD14$^+$CD16$^+$ cells with high expression of HLA-DR, CX_3CR1, and β_2-defensin, which suggests that human CD16$^+$ Mo might migrate from the blood into this inflammatory site. However, we cannot exclude that CD16$^-$ Mo could be their precursors in situ.

The specialized behavior of human Mo subpopulations might be analogous to that reported in mice, where Ly6C^{lo} Mo infiltrated the inflamed peritoneum with *Listeria monocytogenes*; increased the expression of genes associated with detection and removal of microorganisms, inflammatory cytokines, and chemokines; and initiated a program of differentiation into Mϕ. In contrast, Ly6C$^+$ Mo reached the inflammatory site later and began a program of genetic changes related with DC differentiation [23]. It is possible that the mouse Mo subsets had contact with different inflammatory milieus, as they arrived at different time-points into the inflamed peritoneum. Altogether, it would be important to assess the kinetics of migration of human Mo subsets from the blood into inflamed sites. However, this experimental approach was not possible to carry it out in our system, as very few human Mo infiltrated the peritoneum when they were injected directly into the blood. They died rapidly in circulation, leading us to postulate that complement activation by the antibodies used for Mo purification could be responsible for the excessive Mo death. Other authors have shown that total human Mo purified by plastic adherence can migrate from the blood into the peritoneum of *scid* mice by the chemotactic effect of C5a anaphylatoxin or CCL3 and after arrival, acquired a classical DC phenotype only with C5a [49]. The phenotype reported by Soruri et al. [49] with C5a was similar to what we observed, with increased HLA-DR, CD86, and CD83 expression. However, we did not notice decrement of CD14. We are aware that our system lacks Mo extravasation, which might be important for subsequent changes in their differentiation program. Nevertheless, Mo migration through endothelia did not

seem to be responsible for the phenotypical changes reported by Soruri et al. [49].

We obtained a poor recovery of transferred Mo from the peritoneal cavity, which correlated with a high percentage of annexin V^+ cells within the HLA-DR^+ region at early times after transfer. To our best knowledge, there are no reports about this issue with human Mo transferred into mice. However, other studies about murine Mo subsets transferred into congenic mice showed similar or even lower recoveries [2]. One possible explanation is that blood Mo are exceedingly short-lived cells in vitro unless survival factors are provided [11], and our system lacks one of the most critical, M-CSF [50], as murine M-CSF is not active on human cells [51]. Hence, it is plausible that studies about Mo differentiation in the model of *scid-beige* mice could be improved by supplying Mo survival factors, such as human serum or M-CSF. Despite the poor Mo recovery, the Mo subsets have consistently different survival rates in the mouse peritoneum, depending on the inflammatory milieu to which they were exposed. Therefore, the distinct inflammatory environments evoked by zymosan and LPS would contain different cytotoxic and/or survival factors that affect Mo recovery. $CD16^+$ Mo had low survival in the presence of zymosan, although phagocytosis per se did not induce the death of a particular Mo subset. In this condition, $CD16^+$ Mo produced high levels of TNF, which together with the large amount of the endogenous mouse cytokine, could favor the death of this particular Mo subset. In contrast, $CD16^+$ cells had increased survival in inflammatory conditions induced by LPS, and although they produced TNF, the peritoneum had low levels of the mouse TNF. Therefore, the different survival rate could lie, in part, in a distinct sensitivity to the cytotoxic effects of mouse TNF on the human Mo subsets [49, 52]. Alternatively, some chemokines have also been associated with cell death rescue, and CX_3CL1 is particularly related to Mo survival. Our data confirmed that murine CX_3CL1 prevented the death of $CD16^+$ and $CD16^-$ Mo in vitro, as reported by Landsman et al. [11]. Furthermore, the inflammatory milieu led to the production of sCX_3CL1 and to an increase of CX_3CR1 expression in $CD16^-$ Mo in vivo. CCL2 is among the chemokines that promote the expression of CX_3CR1 [53], and as it is induced after LPS and zymosan stimulation, it could be partially responsible for the augmentation of CX_3CR1 expression. The strongest evidence that the CX_3CR1/CX_3CL1 interaction is important for Mo survival in vivo was the blockade of murine CX_3CL1, which promoted human Mo death, although not selectively. This result is in agreement with the nonselective effect of murine sCX_3CL1 on $CD16^+$ and $CD16^-$ Mo survival in vitro [11]. Of note, Mo have improved survival rates in inflammatory conditions compared with homeostasis, which could be related to the increase of CX_3CL1 in the presence of TLR ligands.

In summary, our results foresee a scenario where $CD16^+$ Mo might have immediate microbicidal activity, in agreement with that reported in other studies [18, 23], as they have improved phagocytosis ability and production of inflammatory cytokines and β_2-defensin. Developing $CD16^+$ Mo displayed poor APC abilities, inconsistent with previous data showing that these cells could represent a pool of DC precursors [19, 20]; instead, they might correspond to Mϕ precursors in vivo. In contrast, $CD16^-$ Mo would be preferentially involved in activating the adaptive immune system, concurring with what was reported for their mouse counterparts [2], and become DCs under certain inflammatory conditions.

AUTHORSHIP

S.R.A-R., H.T-A., E.G-D., J.N., and G.G-P. performed experiments. G.V-A. provided patients' peritoneal fluid and monitored their pathological condition. M.A.M-R. and E.A.G-Z. analyzed the results and collaborated with the manuscript discussion. C.S-T. designed the research and wrote the paper.

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KEY WORDS:
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