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Autologous stem-cell transplantation restores the functional properties of CD14+CD16+ monocytes in patients with myeloma and lymphoma

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Abstract: The CD14+CD16+ monocytes appear to be important to immune defense against infection, as these cells are very potent with respect to tumor necrosis factor (TNF) production, phagocytosis, and antigen presentation. Myeloablative high-dose chemotherapy (HDT) and subsequent autologous stem-cell transplantation (ASCT) are being used increasingly for therapy of hematological malignancies, but the pronounced immunosuppression renders the patients prone to infection. To determine the functional properties of CD14+CD16+ monocytes under these conditions, 15 patients with lymphoma or myeloma were examined. Before HDT, the ratio of CD14+CD16+ cells to the population of the classical CD14++ monocytes was 0.28 ± 0.12; this ratio changed during the course of HDT and ASCT in favor of the CD14+CD16+ monocytes to a maximum of 12.4 ± 7.8 ($P<0.001$) on day 3.5 ± 1.6 after transplanation (Tx) and returned to 0.11 ± 0.07 ($P<0.001$) after engraftment on day 11.3 ± 2.2. Although the absolute number of classical CD14++ monocytes declined to less than 1/μl at the nadir, the number of CD14+CD16+monocytes fell from 29.7 ± 9.8/μl to 4.5 ± 3.0/μl at the nadir and increased to 13.8 ± 9.8/μl at the day of discharge from the hospital. Flow cytometric analysis of phagocytosis of fluorescein isothiocyanate (FITC)-labeled *Escherichia coli* showed that 30 ± 10% CD14+CD16+ monocytes of patients were FITC-positive before Tx, and at engrafment, the percentage of FITC-positive cells had doubled to 60 ± 6% (healthy controls, 41±7%). When determining generation of reactive oxygen species after *E. coli* ingestion, the CD14+CD16+ monocytes showed a decreased response before Tx (32±12% positve cells), which increased to 53 ± 24% after ASCT. The median fluorescence intensity of human leukocyte antigen (HLA)-DR expression on the CD14+CD16+ monocytes increased from 11 ± 6 before Tx to 17 ± 11 after Tx, and the production of TNF after lipopolysaccharide showed no remarkable difference (46±13 vs. 49±14 channels). At the same time, expression of TNF and of HLA-DR showed a dramatic decrease in the CD14++ monocytes. Taken together after stem-cell Tx, the function of the CD14++ monocytes is impaired, and the functional properties of CD14+CD16+ monocytes recover, indicating that these cells may be important for defense against infections post-ASCT. *J. Leukoc. Biol.* 75: 207–213; 2004.

Key Words: *phagocytosis · oxidative burst · TNF*

INTRODUCTION

Autologous peripheral stem-cell tranplantation (ASCT), following high-dose chemotherapy, has become a well-established method of treatment for various malignancies [1]. In cases of multiple myeloma (MM) and relapsed non-Hodgkin's lymphoma (NHL), high-dose chemotherapy and ASCT are increasingly becoming the treatment of choice in patients who are less than 65 years old [2]. Major factors concerning morbidity and mortality in these patients before and within the first year after ASCT are infectious complications [3]. Thus, examination of the reconstituted immune system after ASCT is of great importance. Most of the work in that field has focused on the function of B and T cells after ASCT [4, 5], whereas the functional analysis of cells belonging to the innate immunity, e.g., monocytes, has somewhat been neglected. Until now, only little is known about monocyte dysfunction in myeloma and lymphoma and the effect of ASCT on the altered function of these cells. Flieger and Ziegler-Heitbrock [6] found increased levels of several cell-surface molecules on monocytes in chronic lymphocytic leukemia, suggesting an activation of these phagocytes. Dammacco et al. [7] have reported a defect in monocyte chemotaxis in patients with myeloma, and Mainwaring et al. [8]

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have shown impairment of monocyte phagocytosis and killing of *Candida albicans* in myeloma and Waldenström's macroglobulinaemia.

Peripheral blood monocytes play a crucial role in the host response, exerting many different effects. These include antimicrobial and antitumor effects, production of a wide range of cytokines, as well as activation of B and T lymphocytes [9]. Today, it is known that monocytes do not represent a homogenous population of leukocytes but rather can be subdivided in subpopulations with distinct phenotypes and different physiological functions [10]. One approach to define different subtypes of monocytes in peripheral blood is to use monoclonal antibodies (mAb) against CD14 [lipopolysaccharide (LPS) receptor] and CD16 [Fc receptor for immunoglobulin G (IgG) III]. With this method, two populations can be identified [11]: The majority of the monocytes (~90% in healthy donors) show high CD14 expression and lack CD16 expression (CD14++ monocytes), whereas the rest of the cells have a CD14-dim and CD16-bright phenotype (CD14+CD16+ monocytes). The latter population is expanded in various conditions including sepsis, rheumatoid arthritis, and gastrointestinal carcinomas [12–14]. CD14+CD16+ monocytes produce, after challenge with LPS or the bacterial lipopeptide Pam3Cys, significantly higher levels of tumor necrosis factor (TNF) than the CD14++ monocytes [15] but fail to produce any detectable amounts of the anti-inflammatory cytokine interleukin-10 (IL-10) [16]. In fact, it has recently been shown that CD14+CD16+ monocytes are the major source of TNF after stimulation with Pam3Cys [15]. Thus, the minor subset of CD16+ monocytes has been referred to as proinflammatory monocytes. The monocyte subsets also exhibit distinct migration properties in response to chemokines as a result of their different chemokine receptor-expression pattern [17]. Recent work has identified monocyte subgroups in murine peripheral blood based on their expression of the cell-surface chemokine receptor CX3CR1. CX3CR1 high and dim cells seem to correspond to CD14+CD16+ and CD14++ monocytes, respectively [18].

In the present study, we compared functional properties of monocyte subpopulations in lymphoma and myeloma patients with healthy individuals and also examined the effect of myeloablative chemotherapy and ASCT on these different biological functions.

MATERIALS AND METHODS

Patients

Fifteen patients [10 with multiple myeloma, four with NHL, and one with Hodgkin's disease (HD)] were recruited from September 2002 until March 2003 at the Department of Hematology and Oncology of the University Hospital (Munich, Germany). Written informed consent and ethics committee approval were obtained. Eleven apparently healthy members of staff of the university hospital served as controls.

The mobilization regimen consisted of ifosfamide, epirubicin, and etoposide and granulocyte colony-stimulating factor (G-CSF; at a dose of 5 μg/kg body weight). After mobilization, peripheral blood CD34+ stem cells were collected. Myeloma patients under the age of 60 years received high-dose melphalan at a dose of 200 mg/m^2, whereas older patients received melphalan at a reduced dose of 140 mg/m^2.

Reagents

Anti-CD14–fluorescein isothiocyanate (FITC; #6603511), anti-CD14–RD1 (#6603262), anti-human leukocyte antigen (HLA)-DR–PC5 (#IM 2659), and IgG1-PC5 (#IM2663) were purchased from Beckman Coulter (Krefeld, Germany). Anti-CD45–antigen-presenting cell (#MHCD4505), anti-IL-1β–FITC (#MHCIL1B01), mouse IgG1–FITC (#MG101), TNF-α–FITC (#RHTNFA01), rat IgG1–FITC (#R101), and Fix&Perm kit (#GAS-004) were purchased from Caltag (Hamburg, Germany). Anti-CD16–phycoerythrin (PE; #347617) and the phagocytosis and oxidative burst detection kits Phagotest® (#341060) and Phagoburst® (#341058) were from BD Biosciences (Heidelberg, Germany). Brefeldin A (#B 6542) and LPS from S. Minnesota (#L 4641) were from Sigma (Munich, Germany).

Phagocytosis of bacteria

Phagocytic activity was determined by performing a slightly modified version of the protocol described in the Phagotest® kit. Briefly, 200 μl heparinized whole peripheral blood was incubated with 40 μl opsonized FITC-labeled *Escherichia coli* for 10 min at 37°C in a water bath. Samples remaining on ice served as negative controls. To stop phagocytosis, 200 μl Phagotest® quenching solution was added to each sample at the end of incubation time. The samples were then washed with 3 ml Phagotest® washing solution twice and were lysed with 2 ml Phagotest® lysis solution for 20 min at room temperature (RT). After that, cells were washed once again and stained with mAb anti-CD14–PC5 and anti-CD16–PE for 20 min on ice. Monocyte subsets were defined according to their scatter properties and CD14 and CD16 expression. Phagocytosis was determined by detecting FITC-positive cells within the gates of monocyte subpopulations on a fluorescent-activated cell sorter (FACS; Epics XL-MC, Beckman Coulter) using the System II software.

Quantification of oxidative burst activity

Oxidative burst activity was determined by performing a slightly modified version of the protocol described in the Phagoburst® kit. For activation, 200 μl heparinized whole peripheral blood was incubated with 40 μl opsonized *E. coli* for 10 min at 37°C in a water bath. Another sample was incubated with 40 μl Phagoburst® washing solution without *E. coli* and was served as a negative control. For oxidation, 40 μl Phagoburst® substrate solution, containing the fluorogenic substrate dihydrorhodamine 123 (DHR), was added to each sample and incubated for another 10 min at 37°C in a water bath. In the presence of reactive oxygen species (ROS), DHR is converted into a green fluorescent dye. For lysis, the samples were incubated with Phagoburst® lysis solution for 20 min at RT. Cells were washed with Phagoburst® washing solution and stained with anti-CD14–PC5 and anti-CD16–PE for 20 min in on ice. Oxidative burst activity was determined by detecting FITC-positive cells within the gates of monocyte subpopulations.

Intracellular TNF staining

For in vitro culture, peripheral blood mononuclear cells (PBMCs) were prepared from heparinized blood obtained from healthy volunteers and patients by density-gradient separation. Cells (10^6 per ml) were resuspended in 3 ml RPMI 1640 with 10% fetal calf serum (FCS). The samples were incubated for 6 h at 37°C in the presence of the protein transport inhibitor brefeldin A at 10 μl/ml with or without 500 ng/ml LPS. After incubation, cells were stained with anti-CD14–RD1 and HLA-DR–PC5 for 20 min on ice. After that, the cells were fixed with Fix & Perm reagent A for 15 min at RT and were then washed with phosphate-buffered saline (PBS) containing 2% FCS. For permeabilization, samples were incubated afterwards with Fix & Perm reagent B for 15 min at RT. At the same time, anti-TNF–FITC or rat IgG1–FITC was added to the samples. Finally, cells were washed once with PBS, 2% FCS, and were analyzed immediately on FACS. The specific median fluorescence intensity (MFI) was calculated as followed: fluorescence intensity of the specific antibody – fluorescence intensity of the isotype control.

Cell-surface staining and quantification of the ratio of monocyte populations

Whole peripheral blood (200–1000 μl), depending on the absolute leukocyte count, was lysed and twice washed with PBS, 2% FCS. Cells were then stained with anti-CD14–FITC, anti-CD16–PE, and anti-HLA-DR–PC5 for 20 min on

ice. After incubation, cells were washed once in PBS, 2% FCS, and analyzed on FACS.

A gate was first set around monocytes based on their light-scatter properties. Then, two monocyte populations were defined in that gate: the "classical" monocytes being strongly CD14-positive and CD16-negative and the CD14+CD16+ monocytes with a weaker CD14 expression and a bright CD16 coexpression. The ratio of these two populations was calculated by dividing the number of events in the CD14+CD16+ gate by the number of events in the CD14++ gate.

Statistics

For statistical analysis, Student's *t*-test was used. $P < 0.05$ was considered statistically significant.

RESULTS

CD14+CD16+ monocytes during the course of ASCT

Fifteen patients with multiple myeloma or relapsed lymphoma underwent high-dose chemotherapy with subsequent ASCT. The median time between the last chemotherapy regimen and the mobilization regimen was 1.5 months (range, 1–3 months), and the median time from mobilization chemotherapy to ASCT was 2 months (range, 1–14 months). G-CSF was given at a dose of 5 μg/kg body weight after transplantation from day +1 until total leukocyte counts of the patients were greater than 5000/μl. The characteristics of the patients are shown in **Table 1**.

The numbers of monocyte subpopulations in peripheral blood were determined by two or three color-flow cytometry starting on the day of admission to the hospital (=before ASCT, day −8 or −4) to the day of discharge (=after ASCT, day 11.3±2.2). The day of the actual infusion of the autologous stem cells is referred to as day 0.

Before transplantation (Tx), the ratio of the CD14+CD16+ monocytes to CD14++ monocytes in the patients was 0.28 ± 0.11 (n=11), which is comparable with the ratio in healthy controls with 0.26 ± 0.18 (n=11). During the course of high-dose chemotherapy and ASCT, that ratio reversed in favor of the CD14+CD16+ monocytes and reached a peak value of 12.38 ± 7.84 on day 3.5 ± 1.6 ($P<0.0001$). Then, the CD14+CD16+ monocyte ratio again declined with a significant lower mean value of 0.11 ± 0.07 at the day of discharge (day 11.3 ± 2.2) compared with the pre-ASCT ratio ($P<0.001$). Although the absolute number of classical CD14++ monocytes declined to less than 1/μl at the nadir, the number of CD14+CD16+monocytes fell from 29.7 ± 9.8/μl to 4.5 ± 3.0/μl at the nadir only, indicating a higher resistance of these cells to chemotherapy-induced depletion. The CD14+CD16+ monocytes increased to 13.8 ± 9.8/μl at the day of discharge from the hospital (**Fig. 1A**).

Assuming that the CD14+CD16+ monocytes represent a more differentiated type of monocytes that required time to develop, we hypothesized the low number of these cells at the time of discharge may further improve over time. Thus, we examined the monocytes of seven patients that could be followed up several weeks after ASCT. Indeed, the number of CD14+CD16+ monocytes now was 37.5 ± 23.3/μl, and the ratio increased to 0.30 ± 0.18 to a level similar to the pre-ASCT value (Fig. 1B).

Phagocytosis in monocytes before and after ASCT

To assess the impact of Tx on functional properties of monocyte subsets, we measured phagocytosis in each population by flow cytometry using the Phagotest® kit. After phagocytosis of FITC-labeled *E. coli*, cells become FITC-positive in this assay (**Fig.**

TABLE 1. Patient Characteristics

Patient	Sex	Age	Diagnosis	Number of chemotherapy regimens prior to ASCT	Number of reinfused CD34+ stem cells ($\times 10^6$/kg)	High-dose regimen
1	M	22	HD	BEACOPP, BEAM, Gemcitabine, 6 × DHAP	5.83	BuCy
2	M	54	NHL	3 × CHOP	3.46	BEAM
3	F	65	NHL	NOSTE	3.76	BEAM
4	M	53	NHL	4 × FCM	2.86	Cy + TBI
5	F	60	NHL	6 × CHOP	3.73	BEAC
6	F	65	MM	1 × CAD, 3 × VAD	2.03	Mel
7	M	73	MM	1 × ID	7.73	Mel
8	M	66	MM	4 × ID	2.32	Mel
9	M	66	MM	None	3.22	Mel
10	M	64	MM	4 × ID	3.49	Mel
11	M	59	MM	3 × VAD	5.10	Mel
12	F	49	MM	4 × ID	6.93	Mel
13	F	54	MM	4 × ID	2.99	Mel
14	M	58	MM	4 × ID	6.60	Mel
15	M	67	MM	4 × ID	4.99	Mel

M, male; *BEACOPP,* procarbazine, prednisone, cyclophosphamide, doxorubicin, etoposide, vincristine, bleomycin; *BEAM,* carmustine, etoposide, cytarabine, melphalan; *BuCy,* busulphan, cyclophosphamide; *DHAP,* cisplatin, Ara-C, dexamethasone; *CHOP,* cyclophosphamide, doxorubicin, vincristine, prednisone; *F,* female; *NOSTE,* mitoxantrone, prednimustine; *FCM,* fludarabine, cyclophosphamide, mitoxantrone; *Cy + TBI,* cyclophosphamide and total body irradiation; *BEAC,* carmustine, etoposide, cytarabine, cyclophosphamide; *CAD,* cyclophosphamide, doxorubicin, dexamethasone; *VAD,* vincristine, doxorubicin, dexamethasone; *Mel,* melphalan; *ID,* idarubicin, dexamethasone.

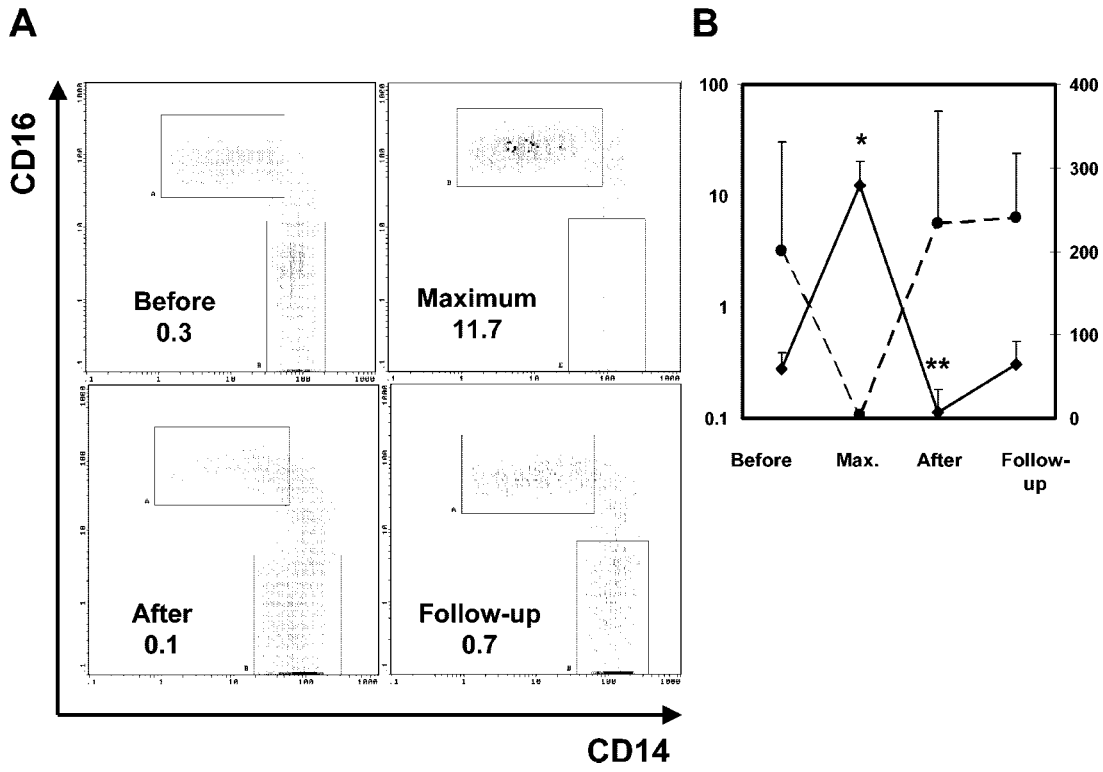

Fig. 1. (A) Whole peripheral blood of patients with lymphoma or myeloma was stained for CD14 and CD16 during the course of high-dose chemotherapy and ASCT. Gates were set around CD14++ (lower gate in the dot plots) and CD14+CD16+ monocytes (upper gate in the dot plots), and the ratio between the event count in the latter and first gate (CD14+CD16+:CD14++) was calculated. Dot plots from a representative patient are shown before chemotherapy (Before, upper left), at the day of the maximum ratio (Maximum, upper right), on the day of discharge from the hospital (After, lower left), and on a follow-up check several weeks later (Follow-up, lower right). (B) The kinetics of the CD14+CD16+:CD14++ ratio on a logarithmic scale (left axis, solid line) and the absolute total monocyte values (cells/μl, right axis, dashed line) are shown for n = 11 patients. Definition of time points described (given as mean±SD): Before, day −4; maximum (Max.), day 3.5 ± 1.6; After, day 11.3 ± 2.2; Follow-up, day 174 ± 111. * and **, $P < 0.01$, versus before value.

2A). Although in healthy subjects, phagocytosis of FITC-labeled *E. coli* is significantly higher in CD14++ monocytes than in CD14+CD16+monocytes (64±7% vs. 41±7%, $P<0.001$), the capability of phagocytosis of CD14+CD16+ monocytes in patients before ASCT is further reduced (30±10%, $P<0.05$ vs. healthy controls). At the same time, the CD14++ monocytes of patients exhibit the same level of phagocytosis (51±18%) as controls. After ASCT, significantly more CD14++ monocytes become FITC-positive (77±10%) within this population. Furthermore, after ASCT, the percentage of FITC-positive CD14+CD16+ monocytes almost doubles to 60 ± 6% ($P<0.001$ vs. before ASCT) and reaches a level higher than healthy controls (Fig. 2B).

Oxidative burst in monocyte subpopulations

We next tested the ability of monocyte subpopulations to produce ROS after ingestion of *E. coli*, using the Phagoburst® kit. As shown in **Figure 3**, in healthy controls, a significantly

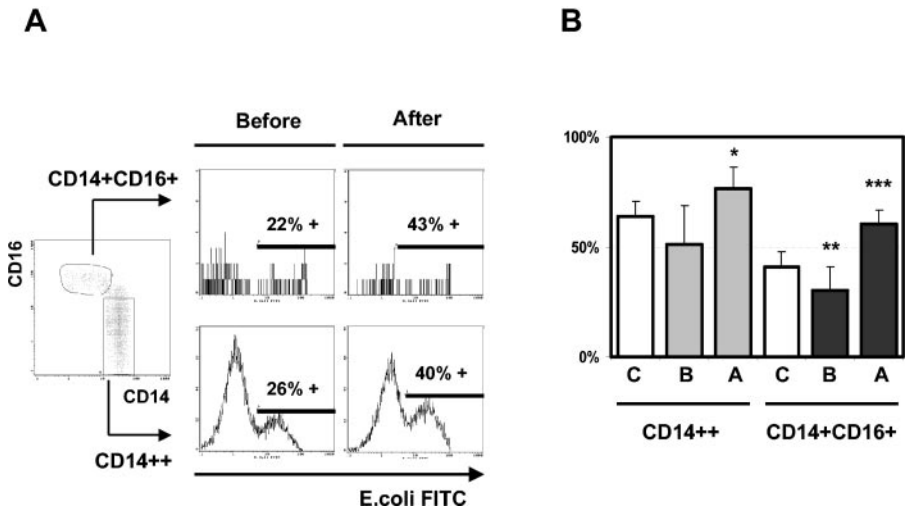

Fig. 2. (A) Whole peripheral blood was incubated with *E. coli* FITC. After incubation, cells were stained for CD14 and CD16. Monocyte subpopulations were then analyzed for phagocytosis of *E. coli* FITC on a flow cytometer. Cells incubated with *E. coli* FITC on ice served as negative control. The solid line in each histogram indicates the gate of FITC-positive cells. Representative histograms from a patient before and after ASCT are shown. (B) Mean values ± SD from n = 6 patients are shown. C, Healthy controls; B, patients before ASCT; A, patients after ASCT. *, $P < 0.05$, versus CD14++ of healthy controls; **, $P < 0.01$, versus CD14+CD16+ of healthy controls; , $P < 0.01$, versus before.

Figure 3

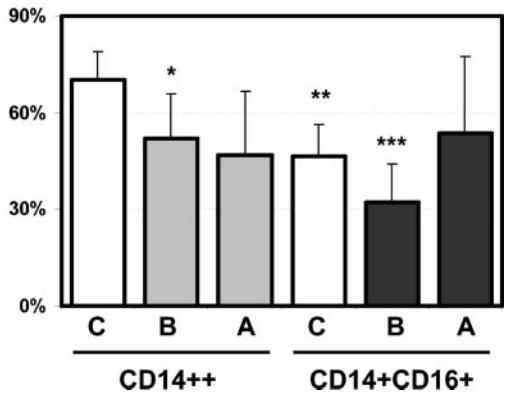

Fig. 3. Whole peripheral blood was incubated with *E. coli*. After addition of a fluorescent dye, the percentage of monocytes positive for oxidative burst was determined on a flow cytometer. Mean values ± SD from n = 6 patients are shown. C, Healthy controls; B, patients before ASCT; A, patients after ASCT. * and **, $P < 0.05$, versus CD14++ of healthy controls; ***, $P < 0.05$, versus CD14+CD16+ of healthy controls.

higher proportion of classical CD14++ monocytes becomes positive for oxidative burst after phagocytosis of *E. coli* when compared with CD14+CD16+ monocytes (70±9% vs. 46±10%, $P<0.05$). In patients, this trend apparently remains, although on a significantly lower level. Although within the CD14++ population, a percentage of 52 ± 14% is positive for oxidative burst, that proportion is reduced to a value of 32 ± 12% among the CD14+CD16+ monocytes. In contrast to this pre-ASCT finding, after ASCT, almost the same percentage of CD14++ monocytes is positive for oxidative burst (47±20%), whereas in CD14+CD16+ monocytes, oxidative burst (53±24%) does not significantly differ from the value in healthy donors any more (Fig. 3).

TNF production and HLA-DR expression remain unaltered in CD14+CD16+ monocytes after ASCT and are strongly reduced in CD14++ monocytes

Previous work has shown that CD14+CD16+ monocytes are a major source of TNF production [15]. Furthermore, it has been shown that CD14+CD16+ monocytes exhibit a higher expression of molecules involved in antigen presentation, such as HLA-DR, than classical CD14++ monocytes [12]. Thus, we also examined the effect of ASCT on TNF production as well as HLA-DR expression in both monocyte populations.

Intracellular TNF production after 6 h of stimulation with LPS in healthy controls confirmed results obtained in previous studies. MFI was significantly higher in CD14+CD16+ monocytes than in CD14++ monocytes (45.7±13.2 vs. 14.7±5.3, $P<0.01$). The level of TNF production of CD14+CD16+ and CD14++ monocytes in patients before ASCT was comparable with controls (61.3±31.9 and 16.7±13.2, respectively, **Fig. 4A**); however after ASCT, although there was no change of TNF expression in the CD14+CD16+ monocytes (MFI, 49.0±13.6), the already-lower MFI in CD14++ monocytes had further dropped by almost 90% to 1.6 ± 1.2 (Fig. 4B).

A similar trend was observed when looking at HLA-DR expression. Before ASCT, CD14+CD16+ monocytes showed a somewhat higher HLA-DR expression than CD14++ monocytes (10.6±5.6 vs. 4.2±2.2, $P<0.01$). Again after ASCT, HLA-DR expression on CD14++ monocytes was reduced by more than 80% to 0.9 ± 0.6, whereas the expression on CD14+CD16+ monocytes showed an increase, although not significant (17.0±10.5, Fig. 4C).

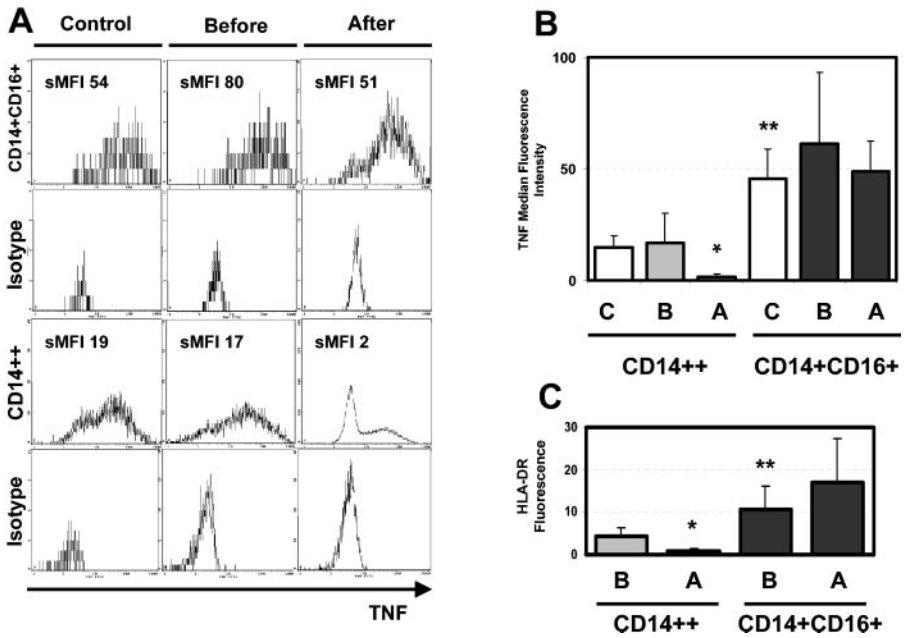

Fig. 4. (A) PBMCs were stimulated with 500 ng/ml LPS for 6 h. After staining for CD14 and CD16 and subsequent permeabilization, cells were stained for intracellular TNF and analyzed on a flow cytometer. Gates were first set around CD14++ and CD14+CD16+ monocytes, respectively, and the TNF expression was measured within each population. The two histograms on the left side show a representative example for TNF expression in a healthy control, whereas the histograms in the middle and on the right show TNF production in a representative patient before and after ASCT. sMFI, specific median fluorescence intensity. (B) MFI of TNF expression is shown. Mean values ± SD from n = 6 patients. C, Healthy controls; B, patients before ASCT; A, patients after ASCT. *, $P < 0.01$, versus CD14++ before ASCT; **, $P < 0.001$, versus CD14++ of healthy controls. (C) Whole peripheral blood was stained for CD14, CD16, and HLA-DR. Fluorescence intensity of HLA-DR expression was then determined for each monocyte population. Mean values ± SD from n = 8 patients. B, Patients before ASCT; A, patients after ASCT. *, $P < 0.01$; **, $P < 0.02$, versus CD14++ before ASCT.

DISCUSSION

Myeloablative chemotherapy and ASCT are now widely used in various hematological and solid malignancies. Especially in multiple myeloma and relapsed lymphoma, the benefits of ASCT in younger patients have been demonstrated. Infections are a major cause of morbidity and mortality among these patients, and immunological dysfunctions in these patients have been described [19, 20]. Thus, several groups have addressed the issue of immune recovery after ASCT, mainly focusing on lymphocyte function [4, 5]. In this work, we have examined CD14++ and CD14+CD16+ monocyte subpopulations during the course of high-dose chemotherapy and ASCT in patients with myeloma and relapsed lymphoma.

We could show that there is no significant difference in the percentage of CD14+CD16+ monocytes in patients and healthy controls. An increased number of CD14+CD16+ monocytes have been shown in solid malignancies [14], but this appears not to be the case in lymphoma and myeloma. Our findings may be a result of prior induction and mobilization chemotherapy. We further observed a decline in the proportion of classical CD14++ monocytes and a corresponding increase of the CD14+CD16+ monocyte fraction, with a peak proportion of more than 98% on days 3–4 after infusion of the autologous stem cells. This dramatic increase in the proportion of CD14+CD16+ monocytes may be a result of a higher resistance to cytotoxic agents compared with CD14++ monocytes. Performing in vitro studies with melphalan, however, we could not show a higher resistance of the CD14+CD16+ monocytes after a 24-h exposure. In fact, the percentage of CD14+CD16+ even decreased (data not shown). Therefore, some unknown factors in the patients' serum might be responsible for the endurance of the CD14+CD16+ monocytes after high-dose therapy. Other possibilities for the increased proportion of CD14+CD16+ monocytes might be the daily administration of G-CSF or reinfusion of high numbers of CD14+CD16+ monocytes in the stem-cell preparation. G-CSF has been shown to induce a CD14+CD16+ phenotype [21], and Tanaka et al. [22] have reported an elevated percentage of CD14+CD16+ monocytes in peripheral blood stem-cell collections. In preliminary experiments with few patients, we could not observe the depletion of monocyte subsets by nonmyeloablative chemotherapy (data not shown).

After engrafment of transplanted stem cells, usually on days 9–10, the increased ratio of CD14+CD16+ monocytes has already reversed to a level below pre-Tx. This finding is in accordance with the concept of CD14+CD16+ monocytes being a more mature monocyte population [12]. Apparently by that time, the pool of peripheral blood CD14+CD16+ monocytes has been depleted through apoptosis or extravasation, and new CD14+CD16+ monocytes have to differentiate yet. This hypothesis is further underlined by the fact that several weeks after ASCT, the percentage of CD14+CD16+ monocytes has returned to baseline levels, suggesting there has been maturation of the classical CD14++ monocytes.

We next addressed the question whether functional properties of monocytes are altered after ASCT. We could demonstrate that in healthy donors, the capability of CD14+CD16+ monocytes to phagocytose *E. coli* and to produce reactive oxygen radicals is significantly decreased compared with CD14++ monocytes. In patients, CD14++ monocytes exhibit similar levels of phagocytosis and significantly reduced levels of oxidative burst compared with CD14++ cells of normal individuals. Although oxidative stress remains unaltered in CD14++ monocytes after ASCT, phagocytosis of *E. coli* is significantly enhanced after Tx. These findings are even more striking when looking at CD14+CD16+ monocytes. We show that in patients, phagocytosis and oxidative burst of CD14+CD16+ monocytes are markedly reduced compared with normal donors. Although after ASCT, the capability to produce ROS is not significantly different from controls anymore, phagocytosis is dramatically improved and doubles the pre-Tx value. Thus high-dose chemotherapy and ASCT obviously not only restore these immunological functions in monocytes but also in case of phagocytosis, even enhance it to supra-normal levels. Dammacco et al. [7] and Mainwaring et al. [8] have reported monocyte dysfunction in myeloma patients, and recently, there have been two reports on functionally defective dendritic cells in myeloma, but none have distinguished between functionally different subgroups of monocytes [23, 24].

The importance of distinction between monocyte subpopulations is further emphasized when looking at cytokine production after LPS stimulation. Baseggio et al. [25] have reported higher secreted TNF protein levels in PBMCs in NHL than in controls, although not significant. In this work, we could confirm these results looking at intracellular TNF production. Furthermore, we show that whereas TNF production of CD14+CD16+ monocytes is comparable in patients and healthy controls, in CD14++ monocytes, TNF production is strongly reduced after ASCT. We have previously demonstrated that CD14++ monocytes account for ~70% of secreted TNF in response to LPS [15]. Thus, our current finding gives evidence of an impaired cytokine response in the majority of monocytes after ASCT. In addition, we can also show an almost complete down-regulation of HLA-DR expression on CD14++ monocytes after ASCT, and in CD14+CD16+ monocytes, HLA-DR expression is slightly increased, although not significantly. The decrease in HLA-DR on the classical monocytes is in line with studies by Reinke et al. [26], who have shown similar effects after immunosuppressive therapy. Although the classical monocytes are impaired, it is important to note that our study shows that the CD14+CD16+ monocytes are still strongly positive and therefore, likely capable of antigen presentation and T cell activation after microbial infection. Although the absolute number of CD14+CD16+ monocytes is low after ASCT, they by far exceed the number of dendritic cells, cells that are also crucial in spite of low frequency.

Taken together, we have shown herein that an initially impaired function of the CD14+CD16+ monocytes in NHL and myeloma will recover after high-dose chemotherapy and ASCT. As the classical CD14++ monocytes at the same time are impaired with respect to TNF and HLA-DR, it might be possible that the CD14+CD16+ monocytes play an important role in antimicrobial defense post-therapy in these patients.

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