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Phagocytic activity of monocytes, their subpopulations and granulocytes during post-transplant adverse events after hematopoietic stem cell transplantation

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ABSTRACT

Phagocytosis of granulocytes and monocytes presents a major mechanism that contributes to the clearance of pathogens and cell debris. We analyzed the phagocytic activity of the peripheral blood cell monocytes, three monocyte subpopulations and granulocytes before and up to one year after hematopoietic stem cell transplantation, as well as during transplant-related adverse events. 25 pediatric patients and young adults (median age of 11.0 years) with hemato-oncological malignancies and non malignancies were enrolled in the prospective study. Ingestion of fluorescence-labeled *Escherichia coli* bacteria was used to assess the phagocytic activity of monocytes and their subpopulations and granulocytes by means of flow cytometry in the patient group as well as in a control group ($n = 36$). During sepsis, a significant increase of phagocytic activity of monocytes ($P = 0.0003$) and a significant decrease of the phagocytic activity of granulocytes ($P = 0.0003$) and the CD14+ CD16++ monocyte subpopulation ($P = 0.0020$) occurred. At the onset of a veno-occlusive disease, a significant increase of phagocytic activity in the CD14++ CD16+ monocyte subpopulation ($P = 0.001$) and a significant decrease in the phagocytic activity of the CD14++ CD16− monocyte subpopulation ($P = 0.0048$) were observed. In conclusion, the phagocytic activity of monocytes, their subpopulations and granulocytes might be a useful and easy determinable parameter that enables identification of post-transplant complications after hematopoietic stem cell transplantation. The alterations of phagocytic activity contribute to the altered immune response that accompanies adverse events after hematopoietic stem cell transplantation.

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Introduction

Post-transplant immune deficiency, extensive immunosuppression and profound granulocytopenia are known risk factors for transplant-related adverse events such as life threatening sepsis, bacteremia, veno-occlusive disease (VOD), and acute graft-versus-host disease (GvHD) after high-dose chemotherapy for hematopoietic stem cell transplantation (HSCT). Little is known about the role of monocytes and their subpopulations, granulocytes and lymphocytes within the immune response after HSCT despite the fact that monocytes perform important functions (such as phagocytic activity) within inborn immunity, as well as within the onset of acquired immunity by releasing pro- and anti-inflammatory cytokines in particular (Calandra, 2003; Cole and Waring, 2002; Matsukawa et al., 2000; Shalekoff et al., 1998). Phagocytosis represents one of the major non-specific defense mechanisms that contribute to the clearing of microorganisms, pathogens and cell debris and might be as well altered in non-infectious conditions (Engelich et al., 2001; Aderem and Underhill, 1999). Various studies have shown that phagocytic activity of leukocytes is altered depending of the illness (Takeda et al., 2011; Prieto et al., 2001; Xu et al., 2012; Voglis et al., 2009; Caradonna

Abbreviations: ANCOVA, analysis of co-variance; CARS, compensatory anti-inflammatory response syndrome; CMV, cytomegalovirus; *E. coli*, *Escherichia coli*; EBV, Epstein-Barr virus; FITC, fluorescein isothiocyanate; GvHD, graft-versus-host disease; HBSS, Hank's balanced salt solution; HSCT, hematopoietic stem cell transplantation; M1, CD14++ CD16− monocyte subpopulation; M2, CD14++ CD16+ monocyte subpopulation; M3, CD14+ CD16++ monocyte subpopulation; SD, standard deviation; VOD, veno-occlusive disease.

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et al., 2000; Li et al., 2010; Lecube et al., 2011; Alvarez-Rodriguez et al., 2013; Dubaniewicz et al., 2012). Also microorganisms and viruses have been shown to induce the activation of granulocytes (Koyama et al., 2000; Haig and McInnes, 2002). The mononuclear phagocyte, or monocyte system, has been postulated in numerous studies as the key to systems of immune defense (Branellec et al., 1982). Phagocytic activity is one aspect of the monocytes' involvement in inherited immunity. Infection with *Escherichia coli* (*E. coli*) is one of the most common causes of sepsis or bacteremia in patients suffering from malignancies (Yilmaz et al., 2009; Katsibardi et al., 2011). Experimental and clinical trials published to date have reported altered phagocytic activity of monocytes and granulocytes during bacterial and viral infection (Danikas et al., 2008). They revealed that decreases in the phagocytic activity of granulocytes and monocytes occurred during sepsis (Hirsh et al., 2001). During compensatory anti-inflammatory response syndrome (CARS), which is known as a immunosuppressed state after the initial hyper-inflammatory phase in sepsis patients, the phagocytic activity in monocytes is significantly reduced being accompanied by a lowered production of the proinflammatory cytokines TNF-α and IL-6 (Xu et al., 2012). Further, it could be shown, that the phagocytic activity of monocytes in mice is supposed to be a main factor in the bacterial clearance during sepsis (Krasnodembskaya et al., 2012) and might be used as a predictor for early-onset sepsis in very low birth weight infants (Hallwirth et al., 2002). By contrast, the phagocytic activity of monocytes and granulocytes are identified as normal in most transplant recipients (Miyagawa and Klingemann, 1997). The subset of CD14+ CD16++ has already been described as a possible GvHD predictor (Rommeley et al., 2011). We hypothesize that phagocytic activity might likewise be altered during adverse events after HSCT. The implications of phagocytic activity of monocytes, monocyte subpopulations and granulocytes within pediatric patients undergoing allogeneic and autologous stem cell transplantation has not to date been examined. To address this issue, we conducted a pilot study and analyzed the phagocytic activity of monocytes and granulocytes before, during and up to one year after HSCT. The aim of the study was to analyze the general phagocytic activity of monocytes, monocyte subpopulations and granulocytes in order to evaluate the role of these cell types before, during, and after HSCT. The present work further addresses the question of whether or not changes arise in the phagocytic activity of monocyte subpopulations during and after HSCT or in cases of post-transplant complications and if these changes might function as a prognostic parameter in adverse events after HSCT.

Materials and methods

This prospective pilot study was conducted in accordance with the declaration of Helsinki and performed with the approval of the University Children's Hospital Tübingen's Institutional Review Board (No.19/2002V). Informed consent was obtained from healthy donors, patients or their legal representatives.

Study design and phagocytosis activity measurements

This prospective single-center study investigates the phagocytic activity of peripheral blood monocytes, their subpopulations, as well as peripheral blood granulocytes in 25 pediatric patients and young adults with hemato-oncological malignancies and non-malignancies before, during and up to one year following autologous and allogeneic stem cell transplantation. The control group was comprised of a total of 36 children and adolescents (<15 years) ($n = 18$) and young adults (>15 years) ($n = 18$). Analyses of phagocytic activity were performed at several points in time: called "baseline", defined as the point in time one day prior to the start of conditioning, during conditioning, and during the post-transplant period. During the post-transplant period analysis of the phagocytic activity in the peripheral blood was conducted every other day from day 0 to day 30 after HSCT, two to three times weekly between day 30 and day 100 after HSCT, one to two times weekly between day 100 and day 200, and once weekly between day 200 and day 365. Upon occurrence of treatment-related adverse events after HSCT, such as sepsis, bacteremia, viremia, acute GvHD or VOD, the analysis of phagocytic activity was performed at least every other day during the course of the event.

Criteria for clinical events and post-transplant complications

The diagnosis of sepsis was performed in accordance with guidelines by the International Sepsis Consensus Conference Pediatric Critical Care 2005 (Goldstein et al., 2005). Bacteremia was defined by at least one positive blood culture. Viremia was defined by the presence of one positive blood sample for cytomegalovirus (CMV), adenovirus, Epstein-Barr virus (EBV), herpes simplex virus, human herpes virus type 6, varicella zoster virus, and Parvovirus B-19 polymerase chain reaction. Localized bacterial and viral infection was diagnosed by evidence of pathogens in the urine, feces, sputum or swab materials. GvHD was defined according to the Glucksberg score (Glucksberg et al., 1974). Diagnosis of VOD ensued according to the Seattle or Baltimore clinical criteria (Jones et al., 1987; McDonald et al., 1984, 1993). A relapse of underlying disease was diagnosed by evidence of leukemic blasts greater than 5% in the bone marrow or based on detection of minimal residual disease greater than 10^{-3}.

Flow cytometric analysis

Fluorescein isothiocyanate (FITC)-labeled opsonized *E. coli* bacteria (US Biological, Swampscott, MA, USA) was resuspended in 0.5 ml of Hank's balanced salt solution (HBSS; PAA, Austria) resulting in a concentration of approx. 2×10^9 bacteria/ml. Two hundred microliters of heparinized whole blood were mixed with 20 μl FITC-labeled *E. coli* and incubated at 37 °C for 10 min. As a control, a sample remained on ice to reduce phagocytosis to a minimum. HBSS was incubated with whole blood cells, as an additional negative control. Phagocytosis was stopped by incubating the samples on ice. External fluorescent signals were quenched by adding 100 μl of 0.5% trypan blue (Biochrom AG, Berlin, Germany). After two washing steps with HBSS, erythrocytes were lysed by adding 2 ml of 10% ammonium chloride solution (0.15 M ammonium chloride, 10 mM potassium hydrogen carbonate, 0.1 mM EDTA disodium salt dihydrate in deionized water) for 5 min on ice. Cells were centrifuged, washed with 3 ml PBS and resuspended in 100 μl 0.5% paraformaldehyde solution for flow cytometric analysis (FACScan, BD Biosciences). To analyze phagocytic activity in granulocytes and monocytes, cells were gated according to their forward- and side-scatter profiles (Fig. 1A).

Percentage of phagocytic activity was then calculated for each cell population and the geometric mean recorded for each time point in relation to all cell populations (phagocytic and non-phagocytic). Percentage of phagocytic activity of monocytes and granulocytes were calculated as:

$$\text{phagocytic activity}\,[\%] = \frac{\text{phagocytic activity of specific cells}}{\text{phagocytic activity of all cells}} \cdot 100\%$$

Phagocytic activity of monocyte subpopulations CD14++ CD16−, CD14++ CD16+ and CD14+ CD16++ were determined by staining cells with PerCP-labeled CD14 (MΦP9) monoclonal antibody (mAb) and PE-labeled CD16 (B73.1) mAb (both Becton Dickinson, Franklin Lanes, NJ, USA). The stained cells were incubated for 10 min in the

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