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Significant Decline of Peripheral Myeloid Dendritic Cells Following Multiple Trauma

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Background. **Dendritic cells (DC) represent an important and integral part of the immune system and are potent initiators of inflammation. Two distinct subsets of DC have been identified: myeloid DC (MDC) and plasmacytoid DC (PDC), which differ widely in many respects. Despite the importance of the DC in the inflammatory response that occurs after severe multiple injury, there is a profound lack of information regarding the distribution and regulation of DC subtypes following multiple trauma. The main goal of this study was to assess whether the normal distribution of circulating DC subpopulations is altered during the first 5 d after multiple trauma.**

Patients and methods. **Sixty-three patients with multiple trauma (ISS 31 ± 15 points) and 11 healthy volunteers (control group) were enrolled. Blood samples were taken on admission (D0) and daily for the following 5 d. The percentages of MDC and PDC were determined by flow cytometry.**

Results. **A significant decline of the MDC concentration was observable on days 3 to 5 after admission in comparison to the values obtained on the day of admission. The ratio of MDC to PDC decreased significantly (3-fold, $P < 0.05$). This reduction correlated significantly with changes observed in the plasma concentrations of IL-10 ($r = 0.5$; $P < 0.05$).**

Discussion. **Our data demonstrate that multiple trauma is followed by a marked change in the subpopulation composition of the DC compartment, and that these changes are inversely associated with enhanced IL-10 plasma concentrations. This imbalance in the DC compartment favoring PDC concentrations may contribute to the immunological alterations that are observed following multiple trauma.**

Key Words: **dendritic cell; myeloid dendritic cell; MDC; plasmacytoid dendritic cell; PDC; multiple trauma; migration, apoptosis; IL-10.**

INTRODUCTION

Multiple trauma is often accompanied by severe immune dysfunction. The initial inflammatory reaction can be triggered by bacterial infections or in response to cellular injury, hypoxia, hypo- and hyperthermia, and reperfusion injury [1, 2]. It has previously been shown that dendritic cells (DC) act as antigen presenting cells and contribute to the initiation of the innate immune response concurrently with the activation of monocytes/macrophages. The activated DC and macrophages are also believed to release various proinflammatory cytokines such as IL-1β, IL-6, TNF-α, and other cytokines including IL-12, IL-15, IL-18, as well as chemokines [3]. Following cytokine expression, reactive oxygen species (ROS) and lipid mediators are released, and cellular adhesion molecules are upregulated. These released mediators often lead to capillary leakage with cell swelling and to a severe disturbance of the coagulation cascade, resulting in the deposition of fibrin clots in small vessel and subsequently to an aggravation of tissue perfusion and organ failure [4]. Concomitant with the onset of inflammation, a more prolonged counter-inflammatory response is initiated that attempts to restore immunological equilibrium and leads to impaired immune function, including the deactivation of macrophages, reduced antigen presentation, T-cell anergy, shift to a T-helper type-2 (Th2) response, and up-regulation of anti-inflammatory mediators (IL-10, IL-1Ra, sTNF-R) [5, 6].

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Dendritic cells are designated as lineage-negative, MHC class II positive, bone marrow-derived mononucleated cells. They are found in tissues throughout the body, particularly those that are exposed to the external environment and where frequent exposure to foreign antigen and micro-organisms occurs, as well as in the circulatory system [7, 8]. In the peripheral blood, two major subsets of DC can be identified based on the expression of the β_2 integrin protein CD11c; first, the myeloid DC (MDC) that consists of two subsets (MDC1, MDC2), and second, the plasmacytoid DC (PDC).

Myeloid DC were characterized by expression of myeloid markers such as CD13, CD33, CD11c, and low expression levels of CD123 [9]. Plasmacytoid DC are derived from a lymphoid lineage and are characterized by CD11c negative and high level of CD123 expressing cells [10, 11]. In addition, PDC stain positive for CD303, a unique lectin protein [12].

In addition to their phenotypic differences, MDC and PDC are also functionally distinctive. They express different patterns of Toll-like receptors (TLR) possess different cytokine receptors and react to an identical stimulus with the release of different cytokines [13, 14].

Despite the potential involvement of DC in the inflammatory response that occurs after severe multiple injury, there is a lack of information regarding the distribution and regulation of DC subtypes following multiple trauma. The main goal of this study was to assess the influence of multiple trauma on the circulating subpopulation of DC and, if changes occur, whether those alterations are associated with either injury severity, demographic data (age, gender), or concentrations of typical trauma related mediators.

PATIENTS AND METHODS

Patients

Patients older than 18 y with an injury severity score (ISS) of 16 points or higher were included. Exclusion criteria included burns, acute myocardial infarction, immunosuppressive therapy, and chronic diseases. We enrolled 63 patients suffering from severe multiple trauma. The mean ISS of male patients ($n = 45$) was 34.1 ± 14.9 points (range: 16–75 points). Their mean age was 41.4 ± 15.9 y (range: 21–75 y). Female patients ($n = 18$) had a mean ISS of 32.6 ± 19 points (range: 16–75 points), their age was 41.2 ± 15.2 y (range: 21–69 y). The first blood sample was obtained on admission of the patient to the emergency room (D0) and then daily over the 5 following d (D1–D5). The average time between trauma and the first blood sampling was 45 ± 18 min. For control values, 11 healthy volunteers (5 female, 6 male, age 30 ± 8 y) were analyzed as well. A SOFA score > 6 points was defined as multiple organ failure. According to the ethical approval of the University of Frankfurt, all patients and healthy volunteers (or their relatives) were required to complete and sign an informed consent prior to enrollment in the study.

Determination of the DC Percentage in Peripheral Blood Mononucleated Cells

The percentage of the DC-fraction of PBMC was assessed using a Blood Dendritic Cell Enumeration Kit (Miltenyi Biotech, Bergisch-Gladbach, Germany) following the instructions of the manufacturer. Patient's blood was consequently handled at 4°C and processed immediately after blood sampling. In brief, PDC, MDC1, and MDC2 were identified by staining each 300 μL EDTA whole blood with anti-BDCA-1-(CD1c)-PE, anti-BDCA-2-FITC (CD303), and anti-BDCA-3-APC monoclonal antibodies, respectively. B-lymphocytes and monocytes as well as defective cells were excluded by simultaneously staining with anti-CD19-PE-Cy5, anti-CD14-PE-Cy5 together with a fluorescent dead cell discriminator reagent. A parallel preparation that served as a control vehicle contained a mixture of all FITC-, PE-, and APC-conjugated isotype monoclonal antibodies. The cells were then washed, fixed with a formaldehyde solution, and finally analyzed using a four color dual laser flow cytometer (FACScalibur, Becton Dickinson, Heidelberg, Germany). A total of 100,000 events were acquired.

For determination of the concentration of DC in the circulation, the percentage of DC of leukocytes was multiplied by the number of leukocytes per μL as assessed by the use of a Coulter-counter.

Plasma Concentrations of IL-6, IL-10

Concentrations of IL-6 and IL-10 in plasma were determined simultaneously using cytometric bead array flex sets (BD-Biosciences, Heidelberg, Germany), following the guidelines of the manufacturer.

Isolation of DC and Assessment of Apoptosis

This experiment aimed to measure the direct effect of the multiple trauma serum on the MDC apoptosis. Therefore, purified DC was used to prevent secondary effects mediated by other cell populations. DCs from buffy coat were isolated using a magnetic separation technique (Blood Dendritic Cell Isolation Kit II; Miltenyi Biotech, Bergisch-Gladbach, Germany). The whole DC isolation procedure was performed at continuously low temperatures (4°C) following manufacturer's instructions. The whole procedure required approximately 2 h. The isolated DC were incubated for 12 h in RPMI-medium (Invitrogen, Karlsruhe, Germany) that was supplemented with pooled serum obtained from multiple trauma patients (20% vol/vol, $n = 10$). Serum obtained on day 2 and, respectively, serum obtained on day 5 following trauma was tested. As a control, pooled serum obtained from healthy volunteers ($n = 5$) was used. Prior to the assessment of apoptosis, the DC were prestained with APC conjugated monoclonal antibodies against CD11c (MDC; BD-

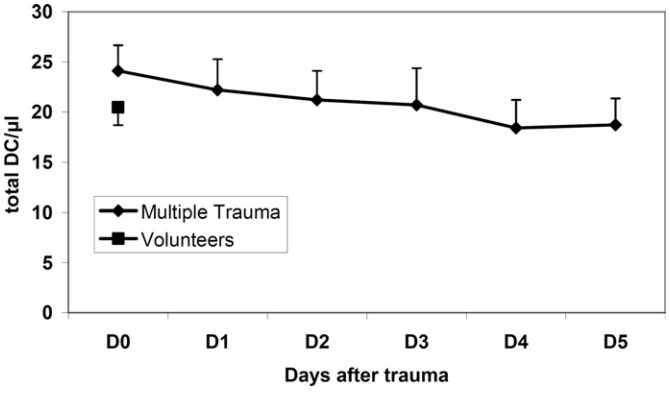

FIG. 1. Concentration of peripheral DC remains unchanged in multiple trauma. The concentration [DC/μL] of total DC in the PBMC fraction during the post traumatic course as calculated by the means of flow cytometry and leukocyte count is shown. Dendritic cells were analyzed in a daily manner; the first sample was obtained in the emergency room. Mean values ± SEM of 63 multiple trauma patients (open circle) and 11 healthy volunteers (filled square) were shown.