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Circulating $CD14^+CD163^+CD115^+$ M2 monocytes are associated with the severity of new onset severe acute pancreatitis in Chinese patients

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ABSTRACT

Background: Despite the role of monocytes in the pathogenesis of severe acute pancreatitis (SAP), it remains unclear how different subtypes of monocytes regulate and contribute to this pathogenesis.

Methods: We examined the numbers of different subsets of monocytes by flow cytometry in 21 SAP, 15 mild acute pancreatitis (MAP) and 13 healthy controls (HC). The concentrations of plasma cytokines were assessed by cytometric bead array. Disease severity was evaluated based on the acute physiology and chronic health evaluation (APACHE) II score and plasma C-reactive proteins (CRP) levels.

Results: Compared with the numbers in MAP patients and HC, we observed that the numbers of $CD14^+CD163^-$, $CD14^+CD163^-MAC387^+$, $CD14^+CD163^-IL\text{-}12^+$ M1 monocytes, and $CD115^+$, $CD204^+$, $IL\text{-}10^+$ M2 monocytes were significantly increased in SAP patients. In addition, these patients showed higher plasma levels of interleukin (IL)-12 and IL-10. Furthermore, the number of $CD14^+CD163^-$, $CD14^+CD163^-MAC387^+$ M1 monocytes and the plasma IL-12 concentration showed a positive association with the CRP level, while the number of $CD204^+$, $IL\text{-}10^+$ M2 monocytes and the plasma IL-10 concentration showed a positive correlation with the APACHE II score. Importantly, the $CD115^+$ M2 subset displayed a positive correlation with both the CRP level and APACHE II score, and treatment of SAP significantly reduced the number of this subset.

Conclusions: The $CD14^+CD163^+CD115^+$ M2 monocyte count appears to be important factor in determining the severity and prognosis of SAP. Both the pro- and anti-inflammatory monocytes appear to participate in the pathogenesis of SAP.

1. Introduction

Severe acute pancreatitis (SAP) is characterized by persistent organ failure (organ failure persisting for ≥48 h) [1], and accounts for 15%–20% of all acute pancreatitis (AP) cases [2]. Gallstone migration and alcohol abuse are the main causes in adults [3]. SAP is characterized by its rapid progression, damage to multiple organs, and high mortality. This high mortality due to SAP has been attributed to immune dysfunction during the early stage and secondary infection along with pancreatic necrosis in the later stage [4–6]. It has been well documented that monocytes/macrophages participate in the immune dysfunction, development and progression of SAP [7–9]. However, little is known about how different subtypes of monocytes regulate the pathogenesis of SAP.

Monocytes typically circulate in the blood and mature into macrophages during inflammation in the tissues [10]. Based on different inflammatory states, macrophages can be classified into activated (M1) and alternatively activated (M2) subtypes [11]. The human monocytes can be converted to M1 subtypes in vitro, by treatment with granulocyte-macrophage colony stimulating factor (GM-CSF) and interferons (IFNs), whereas macrophage colony-stimulating factor (M-CSF), interleukin (IL)-4, and IL-10 treatment leads to production of M2 macrophages [12]. The M1 macrophages produce many pro-inflammatory cytokines, including IL-12 and IL-1β, which have the ability to clear bacteria and tumor cells. Similarly, the M2 macrophages are characterized by expression of anti-inflammatory cytokines (e.g., IL-10),

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which supports tissue repair, tumor growth, and metastasis [13]. The M1 and M2 classifications, which were initially proposed for macrophages, can also be extended to human peripheral blood monocytes [14]. The expression of both M1 and M2 markers is usually detected in circulating peripheral blood mononuclear cells (PBMCs) and can vary in different polarization states of different diseases [14–16]. It has been shown that monocytes/macrophages are the main inflammatory cells involved in the development of early and late SAP [17]. In addition, the relative levels of cytokines IL-10 and IL-12 has also been shown to be valuable tools for the clinical assessment of disease severity [18,19]. However, it is unclear whether the circulating monocyte counts can also aid in evaluating the severity of SAP.

Previous studies have established that monocytes and macrophages play important roles in the pathogenesis of AP, and their activation status can determine the severity of AP [20,21]. We in our earlier study also observed an increase in the $CD14^+CD163^+CD115^+$ M2 monocytes and their association with the plasma C-reactive protein (CRP) level and acute physiology and chronic health evaluation (APACHE) II score in mild acute pancreatitis (MAP) patients, thereby indicating that this subset can be a biomarker for evaluating the severity of MAP [22]. CD115 is a macrophage colony-stimulating factor receptor (CSF-1R) that polarize macrophages toward M2-type [23], and its engagement with CSF-1 is crucial for the survival, differentiation, and possible activation of monocytes/macrophages. CD115 has been widely studied in a variety of human tumors and inflammatory or autoimmune diseases and predicts poor prognosis [11,22,24]. However, the changes in this subset and subsequent association with disease severity along with the expression of other M2 markers have not been fully explored in SAP.

Thus, this lack of information prompted us to characterize the numbers of different monocyte subpopulations in patients with newly diagnosed SAP or MAP and in control subjects. Furthermore, we also assessed the plasma IL-12 and IL-10 levels and the potential associations between different subpopulations and plasma IL-10 and IL-12 concentrations with the CRP level and APACHE II score in SAP patients.

2. Materials and method

2.1. Patients and controls

We recruited 21 SAP patients and 15 MAP patients for our study at the inpatient service of the Department of Gastroenterology, the Second Part of the First Hospital of Jilin University (Changchun, China), between November 2015 and June 2017. All patients met the Atlanta criteria of AP [1], and survived. The APACHE II scores were calculated in these patients to assess the severity of the disease. Based on the Atlanta criteria, there are usually two phases in acute pancreatitis; the early phase and the late phase. The early phase lasts for about one week, while the late phase lasts from weeks to months. In addition, another 13 age-, gender-, and ethnicity-matched healthy controls were recruited from the Physical Examination Center of our hospital during the same period, and all of them had no known inflammatory diseases, autoimmune diseases, or allergies. Even the patients who were on immune-suppressive drugs until 3 months before this study for tumor, autoimmune disease, or any other inflammatory disease, were excluded. The experimental protocol was established according to the Declaration of Helsinki guidelines and was approved by the Human Ethics Committee of Jilin University, the approval number is 2015-213. Written informed consent was obtained from each subject.

2.2. Treatment and follow-up

The SAP treatments included close monitoring, nutritional support, antibiotics, and the suppression of pancreas exocrine secretion. In contrast, the treatment for MAP patients was supportive, including fluid resuscitation, pain relievers, and no oral feeding. All the patients were followed up for 9–11 weeks. We had the complete records of 7 SAP patients, while follow-up information for the other 14 patients was incomplete. The blood samples were collected before and after 9–11 weeks of treatment.

2.3. Clinical examination

The clinical data including age, gender, height, body weight, body mass index (BMI), and information about other laboratory tests was collected from the hospital records for each subject. The full blood cell counts were performed for individual participants, and the concentrations of plasma CRP, amylase (AMY), and lipase (LPS) were analyzed using scatter turbidimetry with a Siemens special protein analyzer (Siemens Healthcare Diagnostics Products, GmbH, Munich, Germany) and ADVIA 1650 biochemical analyzer (Bayer, Pittsburg, PA, USA), respectively.

2.4. Flow cytometric analysis

Heparinized fasting venous blood samples (6 mL) were collected from the median cubital vein of each SAP or MAP patient (within 72 h after upper abdominal pain occurred, and during the first 24–48 h after admission) and HCs, and then PBMCs were isolated by density-gradient centrifugation using Ficoll-Paque Plus (Amersham Biosciences, Little Chalfont, UK). PBMCs at a concentration of 1×10^6 cells/tube were stained in duplicate with BV510-anti-CD14 (BD Biosciences), PE-anti-CD115 (BD Biosciences), PE/Cy7-anti-CD163 (Biolegend), APC/Cy7-anti-206 (Biolegend), and APC-anti-204 antibodies for 30 min in the dark at 4 °C. Subsequently, the PBMCs were washed twice and then processed using a fixation/permeabilization kit (BD Biosciences) for fixation and permeabilization. This was followed by intracellular staining with FITC-anti-MAC387 (Abcam) antibody.

For functional assays, we stimulated PBMCs (10^6 cells/well) in duplicate with 50 ng/ml lipopolysaccharide & phorbol myristate acetate (PMA), and 1.0 μg/ml of ionomycin (Sigma-Aldrich, St. Louis, USA) in complete RPMI1640 media for 2 h in 5% CO_2 at 37 °C. PBMCs were further exposed to Brefeldin A (GolgiPlug; BD Biosciences) for 4 h, as described previously [25,26]. After washing, these PBMCs were stained with BV510-anti-CD14 (BD Biosciences) and PE/Cy7-anti-CD163 (Biolegend) antibodies, and later fixed and permeabilized using the permeabilization solution (BD Biosciences). This was followed by intracellular staining with BV421-anti-IL-12 (BD Biosciences) and PE-CF594-anti-IL-10 (BD Biosciences) antibodies. The fluorescence minus one (FMO) method was used to distinguish the positive population of PBMCs from the negative population. Finally, the percentages of different subpopulations of monocytes were characterized using a FACSAria II (Beckton-Dickinson), and the data were analyzed with FlowJo software (v5.7.2, TreeStar Inc., Ashland, OR, USA).

2.5. Cytometric bead array (CBA) analysis of serum cytokines

The plasma concentrations of IL-12 and IL-10 were determined by the CBA method, according to the manufacturer's protocol (BD Biosciences) with minor modification. Briefly, each plasma sample (50 μL) in duplicate was used to detect the plasma concentrations of IL-12 and IL-10 in all participants, using a CBA kit on a FACSAria II (Beckton-Dickinson) machine. The plasma concentrations of cytokines were quantified using CellQuestPro and CBA software (Becton Dickinson). The detection limits for IL-12 and IL-10 in this method were 1.9 pg/ml and 3.3 pg/ml, respectively.

2.6. Statistical analysis

All data are expressed as median and range. The differences between groups were analyzed by Kruskal-Wallis test. The differences between pre-treatment and post-treatment values were analyzed using Wilcoxon test. However, the relationship between variables was