Clinical Investigations

Monocyte Subsets and Inflammatory Cytokines in Acute Decompensated Heart Failure

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ABSTRACT

Background: Distinct monocyte subsets predict cardiovascular risk and contribute to heart failure progression in murine models, but they have not been examined in clinical acute decompensated heart failure (ADHF).

Methods and Results: Blood samples were obtained from 11 healthy control subjects (HCs) and at admission and discharge from 19 ADHF patients. Serologic markers of inflammation were assessed at admission and discharge. Monocyte populations were defined with the use of flow cytometry for cell-surface expression of CD14 and CD16: CD14++CD16− (classic), CD14++CD16+ (intermediate), and CD14+CD16++ (nonclassic). In ADHF patients, C-reactive protein (CRP) and interleukin-6 (IL-6) were higher compared with HCs (both $P < .001$) and decreased from admission to discharge (CRP: 12.1 ± 10.1 to 8.6 ± 8.4 mg/L [$P = .005$]; IL-6: 19.8 ± 34.5 to 7.1 ± 4.7 pg/mL [$P = .08$]). In ADHF patients, the admission proportion of CD14++CD16− monocytes was lower (68% vs 85%; $P < .001$) and that of CD14++CD16+ (15% vs 8%; $P = .002$) and CD14+CD16++ (17% vs 7%, $P = .07$) monocytes higher compared with HCs. Additionally, the proportion of CD14++CD16− monocytes increased (68% to 79%, $P = .04$) and the CD14+CD16++ monocytes decreased (17% to 7%, $P = .049$) between admission and discharge.

Conclusions: Following standard treatment of ADHF, the monocyte profile and circulating inflammatory markers shifts to more closely resemble those of HC, suggesting a resolution of the acute inflammatory state. Functional studies are warranted to understand how specific monocyte subsets and systemic inflammation may contribute to ADHF pathophysiology. (*J Cardiac Fail 2016;22:358–365*)

Key Words: Heart failure, innate immunity, monocytes, inflammation.

More than 5 million Americans currently have heart failure (HF), and acute decompensated heart failure (ADHF) is the most common reason for hospital admission among persons older than 65 years.[1] With the increasing incidence of HF risk factors (eg, age, obesity, and diabetes), ADHF hospitalizations are likely to increase in the coming years. More than one-third of patients who survive ADHF hospitalization are rehospitalized or die within 90 days of initial discharge,[2] and 1-year mortality is >30%.[3] Unfortunately, current therapy for ADHF is empiric and largely based on expert consensus.[4,5]

Abnormalities in the inflammatory cascade have been associated with both the initiation and the progression of HF.[6] The detrimental consequences of sustained inflammation in HF models led to several controlled trials aimed at neutralizing proinflammatory cytokines in HF patients. However, overall results were neutral in those studies, and an appreciable number of patients had worsening of HF,[7,8] raising

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important questions about how the immune system contributes to ADHF. The innate immune system could modulate the inflammatory component of HF through several mechanisms, including the production of inflammatory cytokines (eg, tumor necrosis factor [TNF] α, interleukin [IL] 6) and reactive oxygen species, activation of the complement system, and modulation of endothelial cell function and myeloid cell recruitment.

Monocytes are key innate immune system mediators of inflammatory responses. Monocyte dysregulation has been implicated in the pathogenesis of diverse diseases, including diabetes, tumor metastasis, pulmonary fibrosis, myocardial infarction, and atherosclerosis. Functionally distinct monocyte subsets can be characterized with the use of flow cytometry for surface expression of FcγIII receptor CD16 and the lipopolysaccharide receptor CD14. The Nomenclature Committee of the International Union of Immunological Societies defines 3 subsets by this method: CD14++CD16– (classic), CD14++CD16+ (intermediate), and CD14+CD16++ (nonclassic).[9] The distribution of these monocyte subsets changes with age[10] and affects the risk for cardiovascular events even if clinically evident vascular disease is not present.[11,12] Recently, Ismahil et al found that differential monocyte subpopulations causally contribute to disease pathogenesis in a murine model of HF,[6] but data in human HF are sparse and cross-sectional.[13,14] We sought to identify changes in monocyte subsets over the course of HF hospitalization, because this information could suggest further avenues for the study of ADHF pathophysiology.

Methods

Study Design

The goals of this study were to compare serologic inflammatory markers and monocyte subsets between ADHF patients and healthy adult control subjects (HCs), and to determine how these parameters change over the course of hospital treatment for ADHF. For the purposes of this study, 3 monocyte subsets were considered: CD14++CD16– (classic), typically accounting for 80%–90% of cells in the circulation, and the CD14++CD16+ (intermediate) and CD14+CD16++ (nonclassic) subsets, which account for the remaining 10%–20%.[9] Because changes in monocyte subsets during ADHF have not previously been reported, and few studies have examined differences between HF patients and HCs, we powered the study based on IL-6, a marker of systemic inflammation. Based on a prior cross-sectional study of symptomatic HF outpatients vs HCs, we predicted a decrease in plasma IL-6 from 40 to 15 pg/mL between admission and discharge with standard deviation of ±25 pg/mL and mean IL-6 of 3.0 pg/mL in HCs. With the use of these assumptions, ≥20 ADHF subjects and ≥5 HCs were thought to be sufficient to demonstrate significant between-group and pre-post ADHF hospitalization differences in plasma IL-6 levels at $\alpha = 0.05$ and $\beta = 0.20$.

Enrolled Patients

Patients hospitalized at the University of Michigan Health System with an admitting diagnosis of ADHF were eligible for this study. Baseline exclusion criteria were acute coronary syndrome, severe renal dysfunction (dialysis or estimated glomerular filtration rate <15 mL min^{-1} 1.73 m^{-2}), cirrhosis or other evidence of severe hepatic dysfunction, active infection or inflammatory disorder (eg, rheumatoid arthritis, lupus), active smoking, active solid organ or hematologic malignancy, use of immunosuppressive therapy (including corticosteroids), and planned mechanical circulatory support, cardiac transplantation, or other surgical procedure during the index hospitalization. Patients who were found to have active infectious or inflammatory conditions (eg, pneumonia, gout flare) after initial enrollment and those who had surgical procedures, blood product transfusion, or immunosuppressive therapy during hospitalization were excluded from the analysis. Nonsmoking older adult (aged ≥65 y) HCs were recruited with the use of a database managed by the University of Michigan Claude D. Pepper Older Americans Independence Center.

Sample Collection and Analysis

ADHF patients with an anticipated hospital length of stay >48 hours were enrolled and admission/baseline blood samples drawn within 24 hours of hospital admission. Discharge blood samples were drawn on the date of hospital discharge, or within 24 hours before an anticipated weekend discharge according to laboratory facility availability. Blood samples were drawn from HC subjects at a single baseline visit.

Analyses for basic laboratory parameters including renal function, electrolytes, high-sensitivity C-reactive protein (hs-CRP), and complete blood count with differential were performed by the University of Michigan Pathology Laboratory with the use of standard techniques. The University of Michigan Cancer and Immunology Core Laboratory measured plasma IL-6, IL-10, TNF-α, and transforming growth factor (TGF) β with the use of commercially available high-sensitivity Luminex assays (EMD Millipore, Billerica, Massachusetts) and soluble CD-14, monocyte chemotactic protein (MCP) 1, and Regulated on activation, normal T expressed and secreted (RANTES) levels with the use of a commercially-available enzyme-linked immunosorbent assay.

Monocyte Isolation and Flow Cytometry

Monocyte populations were analyzed in all patients and assessed for expression of CD14 and CD16 to define the circulating monocyte populations. Eight milliliters of whole blood was collected by venipuncture in a Cell Preparation Tube (CPT) With Sodium Citrate (BD Vacutainer, Franklin Lake, New Jersey) for each study participant at the time points outlined above. The CPT tubes were centrifuged at 2000*g* for 30 minutes, inverted 5×, and stored overnight. The next day, the top layer was removed, split into 2 × 4–mL aliquots, diluted up to 12 mL with ice cold phosphate-buffered saline solution (PBS) to wash, and centrifuged at 300*g* for 10 minutes.