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Letter

RESEARCH CORRESPONDENCE

Acute Ischemia Alters Human Pericardial Fluid Immune Cell Composition

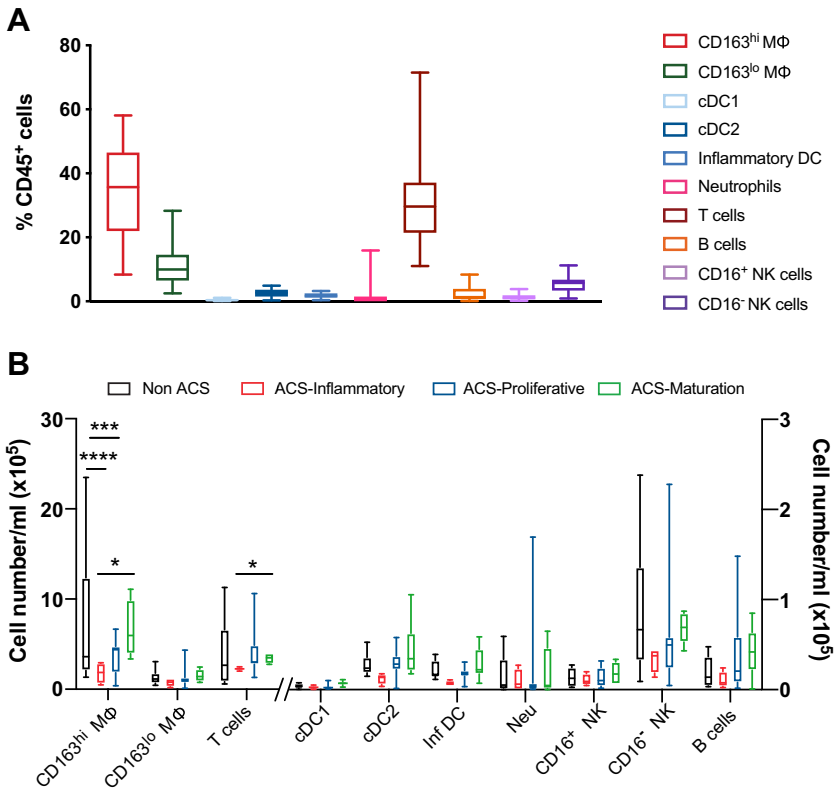
The human pericardial space is an enclosed cavity that provides a homeostatic environment for optimal physiologic functioning of the heart. The contents of this space include pericardial fluid (PF), which is a highly enriched milieu of cytokines, growth factors, and cardiac hormones. These bioactive agents regulate cardiac function through various actions and exhibit dysregulation in different disease states (1). The localization and slow turnover rate of PF make it a potentially powerful diagnostic biomarker for cardiac diseases. The interplay between PF and myocardium can also inform the design of future therapeutic interventions that can facilitate precision medicine. However, the clinical translation of this poorly explored area has been limited by a lack of interest and the use of old experimental techniques. Our team recently discovered a population of immune cells, Gata6-expressing macrophages, to populate the pericardial space (2). In an experimental myocardial infarction model, we found that these resident pericardial macrophages rapidly relocate to the heart and help mitigate adverse cardiac fibrosis. Human pericardial immune cells may contribute to similar clinical benefits. To date, however, no study has comprehensively evaluated the immune cell composition of human PF following acute coronary events. For the first time, we characterize the PF immune cell profile of patients undergoing cardiac surgery.

PF was collected from patients (n = 30) undergoing elective coronary artery bypass grafting surgery (acute coronary syndrome [ACS] group, n = 21) and valve replacement or repair surgery (non-ACS group, n = 9). To mimic the stages of tissue healing post-injury, patients undergoing coronary artery bypass grafting surgery were recruited based on days post-diagnosis of an ACS: inflammatory phase, surgery was done <4 days from the event (n = 4); proliferative phase, surgery was done between 4 and 14 days from event (n = 13); and maturation phase, surgery was done after 14 days from event (n = 4). Patients undergoing valvular surgery presented with aortic stenosis (n = 7) or mitral regurgitation (n = 2). The study protocol was approved by the University of Calgary (Calgary, Alberta, Canada) Institutional Research Ethics Board, and informed consent was obtained from all patients.

The demographics of the ACS and the non-ACS groups are summarized in Supplemental Table 1. In all cases, PF was collected at the beginning of the surgery prior to administration of systemic steroids or the institution of cardiopulmonary bypass. Immediately following collection, immune cell populations were evaluated by flow cytometry using a 13-color panel. We found 2 macrophage populations, 2 classical dendritic cell populations, inflammatory dendritic cells, neutrophils, T cells, B cells, and 2 natural killer cell populations (**Figure 1A**). $CD163^{hi}$ macrophages and T cells were the most represented populations accounting for over 60% of total immune cells. Gata6 expression evaluation revealed that the $CD163^{hi}$ macrophage population represented the main Gata6-expressing myeloid cell population within the PF. Notably, compared with non-ACS control subjects, ACS patients in the early inflammatory and proliferative phases displayed reductions in $CD163^{hi}$ macrophages (**Figure 1B**). Both $CD163^{hi}$-macrophages and T cells showed similar increases in cell numbers between the inflammatory and maturation phases (**Figure 1B**). Interestingly, this mimics a similar pattern for Gata6-macrophages following experimental myocardial infarction. Further analysis revealed that $CD163^{hi}$ macrophage numbers correlate with the timing following the coronary event ($r = 0.5961$; $P = 0.0043$). This can potentially be an important predictive clinical marker that differentiates between disease states in patients presenting with an ACS.

As a homeostatic niche, the pericardial space can be a good representation of cardiac health. Our current understanding of PF is based on routine hematological, biochemical, and cytological tests performed on patients presenting with conditions that necessitate entry into the pericardial space. Butts et al (3) showed that some proinflammatory

FIGURE 1 Pericardial Fluid Immune Cell Profiles From ACS and Non-ACS Patients



(A) Proportional quantification of immune cell populations within patient pericardial fluid (n = 30). **(B)** Quantification of pericardial immune cell numbers from non-acute coronary syndrome (ACS) (n = 9) and ACS patients collected during the inflammatory phase (<4 days; n = 4), during the proliferative phase (4-14 days; n = 13), or during the maturation phase (>14 days; n = 4) following the acute coronary event. Two-way analysis of variance with Tukey's post hoc test was performed for statistical analysis. *P = 0.05. ***P = 0.001. ****P = 0.0001. CD = cluster of differentiation; cDC = classical dendritic cell; DC = dendritic cell; Inf DC = inflammatory dendritic cell; Mϕ = macrophage; Neu = neutrophil; NK = natural killer.

markers remain elevated in the thoracic fluid of patients post-cardiac surgery. This likely represents the impact of the surgical-associated injury. In contrast, the current study collected samples at the beginning of operation and thus reflects the impact of the individual cardiac states on PF immune cell composition. Surprisingly, despite changes in Gata6 macrophages and T cells, pericardial fluid from ACS patients did not demonstrate a recruitment of neutrophils and monocytes that are typically observed in the infarcted heart. This would suggest that the pericardial environment does not simply mirror the cardiac environment. Further exploration of pericardial immune cell fate following cardiac injury is needed, including evaluating potential interactions with pericardial lining or possible relocation (eg, heart, lymph node) that could account for the observed changes. Future work is also required to delineate how these immune cell populations influence the PF molecular profile, and to ultimately determine their possible contributions to cardiac homeostasis and repair. In the era of precision and personalized medicine, a better understanding of PF immune cell function may help inform pericardial targeted strategies to attenuate pro-remodeling inflammatory pathways.

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The authors attest they are in compliance with human studies committees and animal welfare regulations of the authors' institutions and Food and Drug Administration guidelines, including patient consent where appropriate. For more information, visit the Author Center.

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APPENDIX For a supplemental table, please see the online version of this paper.