Published in final edited form as:

Crit Care Med. 2015 July ; 43(7): 1532–1534. doi:10.1097/CCM.0000000000001046.

THE MONOCYTE THAT WASN'T

Philip A Efron, MD, FCCM, FACS and Alicia M. Mohr, MD, FCCM, FACS

Department of Surgery, University of Florida College of Medicine, Gainesville, FL

Keywords

monocyte; sepsis; human; tumor necrosis factor-alpha converting enzyme (TACE); ADAM-17; systemic inflammatory response syndrome (SIRS); myeloid derived suppressor cells (MDSCs)

This issue of *Critical Care Medicine* has a feature article entitled “*Monocyte tumor necrosis factor-alpha converting enzyme catalytic activity and substrate shedding in sepsis and non-infectious systemic inflammation*” (1). The authors eloquently demonstrate in a human translational study that monocyte tumor necrosis factor-alpha (TNF) converting enzyme (TACE) activity is altered by sepsis. This was displayed without an alteration in its cell surface expression, suggesting it was due to a difference in function after sepsis rather than a decrease in the amount of TACE. In addition, this phenomenon was not seen with the systemic inflammatory response syndrome (SIRS). The authors’ work revealed an increase in TACE basal enzymatic cell surface activity in isolated monocytes from septic patients, which was shown in part by an attenuation of TNF receptor shedding. This research also indicated that these monocytes have an attenuated p38-MAP-kinase (MAPK) response, which in part could explain the mechanism behind the changes in TACE activity seen with sepsis. The authors reveal that this change in TACE function after sepsis could be recapitulated using a two-hit in vitro model to imitate sepsis, using the pathogen-associated molecular pattern (PAMP) lipopolysaccharide (LPS). These results led the authors to speculate that PAMPs could be the cause of this change in TACE function after sepsis. The monocyte alterations were not displayed with SIRS patients, and this is supported by the fact that PAMPs would not be as prevalent or present at all in the circulation with SIRS (ex. trauma, pancreatitis, etc.).

TACE was first identified in 1997, and is also known as ‘a disintegrin and metalloproteinase’ (ADAM-17) (2). It is a zinc-dependent modular cell surface protein that closely resembles Class III snake venom enzymes (2). Although it was first described as the enzyme responsible for cleaving membrane-associated TNF- α and driving its appearance in plasma (2), TACE has also been determined to be a crucial enzyme for ectodomain shedding, acting on a large number of substrates related to inflammation (3, 4). This

Direct correspondence to: Philip A. Efron, MD, FCCM, FACS, Department of Surgery, University of Florida College of Medicine, PO Box 100108, Gainesville, Florida 32610, Philip.Efron@surgery.ufl.edu, Phone: (352) 265-0494, Fax: (352) 265-0676.

Copyright form disclosures: Dr. Efron consulted for MERCK (Sepsis Scientific Input Engagement), lectured for the Sinai Hospital of Baltimore/Johns Hopkins University (Visiting Professor) and received support for article research from the National Institutes of Health (NIH) (R01 GM40586-24; R01 GM-081923-06; P30 AG028740; 1 R01 GM113945-01; P50 GM111152-01). His institution received grant support from the NIH/NIGMS.

includes, but is not limited to, activation of TNF receptors (R) and IL-6R-mediated signal transduction (4).

The strengths of this translational study include its novel findings and its clinical applicability in intensive care unit (ICU) patients. The patients studied were classified under the current definition of severe sepsis (69%) or septic shock (31%) (5). One of the biggest clinical dilemmas for the intensivist is determining whether a new critically ill patient's condition is related to infection or not, as the signs, symptoms, and organ dysfunction of both are very similar. With infectious conditions, delayed antibiotic treatment can worsen the patient's outcome (6); whereas with non-infection, giving inappropriate antimicrobial therapy to patients risks inducing bacterial antibiotic resistance as well as altering the patient's microbiome, which may also affect outcome (7). The authors describe a conceivable future diagnostic assay to aid the intensivist. Monocytes are an easy cell to isolate from the patient's blood, thus a rapid test to determine TACE function after the patient's admission to the ICU has great potential to significantly impact clinical practice. In addition, the authors demonstrated that TACE activity correlated well with the severity of illness. Therefore, an examination of a patient's monocytic TACE function could be added to other predictive measures used to help determine a patient's long term outcome from sepsis, as well as determine if immunomodulatory therapies should be used to improve the patient's outcome to severe infection (8–10).

The main limitation of the study was its small sample size. This limits a definitive correlation of TACE activity with mortality. In addition, the small sample size makes it difficult to determine if other factors such as age may have acted as a confounder. Sepsis is a disease of the elderly (11), and the authors found a significant difference in the age of their sepsis patients as compared to their SIRS patients. Finally, caution should be exercised in the interpretation of the results as the cell surface markers used to define monocytes were not specific enough to guarantee that those were indeed the circulating leukocytes being analyzed by the investigators.

For many years now, researchers have been trying to explain the immune dysfunction induced by sepsis through monocyte defects in circulating monocyte function (10, 12). Specifically, the terms “monocyte deactivation or reprogramming” were listed by the authors. O’Callaghan et al. concluded from their work that it is more likely a “resetting” of the monocyte phenotype rather than “simple deactivation.” However, there may be an alternate reason that explains the known immune dysfunction that occurs with sepsis, and this reason is consistent with the authors’ ex vivo results. Since the authors’ definition of what constitutes a monocyte was not sufficiently precise, they may be describing a heterogeneous population of CD11b⁺CD14⁺CD56[−] myeloid leukocytes consisting in part of ‘myeloid derived suppressor cells (MDSCs)’.

MDSCs are a well-known immature myeloid cell population described in the cancer literature, but are a relatively novel population to sepsis investigators (12–15). MDSCs consist of a heterogeneous subset of immature myeloid cells whose numbers increase dramatically after inflammation. MDSCs are potentially immunosuppressive while simultaneously contributing to low grade, chronic inflammation (12–15). Researchers are

readily starting to identify this cell type after infection (14, 18). During sepsis, many circulating monocytes are activated and recruited from the blood to sites of infection, differentiating into macrophage populations within 12 hours (14, 18). Thus, by the time the authors isolated their leukocytes within 48 hours of ICU admission, many monocytes may have already migrated from the blood and been replaced through emergency myelopoiesis with circulating MDSCs (13–15).

The authors utilized Histopaque -1077 (Sigma) in conjunction with Leucosep tubes (Greiner, Stonehouse, UK) or a CD14⁺ magnetic bead selection strategy (Miltenyi Biotech, Surrey, UK) to isolate their CD14⁺ peripheral blood mononuclear cells. However, these steps would not have excluded the enrichment of monocytic MDSCs, which are known to also show varying degrees of CD14 expression (13, 20). Another important point was that the authors were unable to analyze their isolated leukocytes for CD16 cell surface expression, which can help differentiate between monocytic MDSCs and monocytes (13, 20). The authors defined monocytes as CD14⁺CD11b⁺CD56⁻; but immature monocytic MDSCs can share that exact same cell surface marker phenotype (20)! In septic patients from the study, the isolated cells expressed low levels of HLA-DR while having increased basal levels of ADAM17. Decreased expression of HLA-DR is one of the hallmarks of MDSC expression, and ADAM17 is one of the proteins through which MDSCs induce immunosuppression (13, 15, 22). Other researchers have determined that by blocking myeloid cell differentiation through p38 MAP-kinase dependent pathways, post-inflammatory infection was increased (23), again similar to the results determined by O'Callaghan et al.

Objectively, MDSCs would not explain the authors' in vitro results, as the authors isolated monocytes from healthy controls and there are very few MDSCs circulating in healthy patients (14, 16–18). However, the authors did recognize that there are limitations in relating their two-hit model LPS model of healthy volunteer monocytes to that of in vivo circulating leukocytes after sepsis. In addition, it is likely that the monocytes undergoing chronic exposure to a toll-like receptor agonist (ex. LPS) may well be on their way to a more 'macrophage-like' phenotype rather than representing the circulating leukocytes typical of a septic patient.

In May of 1975, Isaac Asimov published an essay, *The Planet that Wasn't* (24). Among other things, it detailed the scientific search for the hypothesized planet "Vulcan," which was supposed to exist between the planet Mercury and the Sun (24). Contemporaries at the time erroneously concluded that Vulcan existed because it was the only feasible explanation, using Newtonian physics, to justify the peculiar orbit of Mercury (24). It wasn't until Einstein presented his General Theory of Relativity, which could account for Mercury's orbit, that the existence of Vulcan could be disproved (24). Although not a perfect analogy, the current search for the mechanism behind dysfunctional monocytes in sepsis has fostered many hypotheses, which will likely evolve as our knowledge of emergency myelopoiesis expands. Although immune dysfunction in sepsis is assuredly complex with multiple pathways, the prime cause may be related to a patient's creation of a different stem cell-derived leukocyte population rather than being related to the host altering the function of

their existing circulating cell populations. This in turn, may impact how sepsis immunotherapy would be conducted in the future.

Acknowledgments

Sources of Support: This work was supported by grants R01 GM40586-24 and R01 GM-081923-06 awarded by the NIGMS. In addition, PAE was supported by P30 AG028740 from the National Institute on Aging and by the NIH NIGMS grant 1 R01 GM113945-01 and by P50 GM11152-01 (NIGMS). Also, AM was supported by NIH NIGMS grant R01 GM105893-01A1.

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