PNAS COMMENTARY

Dietary modification of myeloid-derived suppressor cells (MDSC) activity in sepsis

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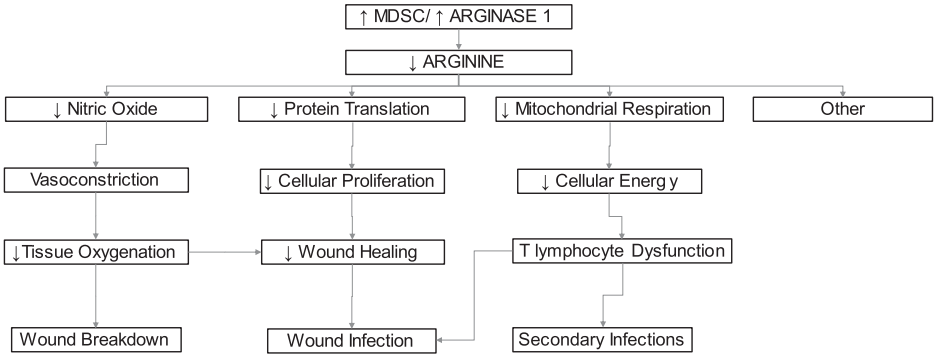

Fig. 1. ADS is characterized by a decrease in arginine availability resulting in a cascade of impaired biochemical processes and altered organ physiology, ultimately leading to clinical manifestations of illness. ADS is observed in different illnesses such as cancer, after physical injury (trauma/surgery), hemolytic illnesses, and possibly in certain infections and potentially in sepsis.

Sepsis, defined as the systemic effects secondary to an infection, is a frequent reason for admission to the intensive care unit, affecting millions of patients worldwide and causing significant morbidity and mortality (1). It has been frustratingly difficult to make progress in the management of sepsis, and currently the best approaches have had to settle with commonsense measures such as early antibiotics, the use of intravenous fluid resuscitation, and use of vasopressors. Innovative approaches in patients with bacterial sepsis, such as the modulation of immune responses targeted at blocking endotoxin or the production of inflammatory cytokines such as tumor necrosis factor, have for the most part failed. Reizine et al., in an article in PNAS, take a different approach (2).

While an initial "cytokine storm" and an uncontrolled inflammatory response is central to our understanding of the pathologic immune responses, sepsis is also associated with alteration in T lymphocyte subsets and impaired T lymphocyte function. This is called by different names, including "immune paralysis" or "anergy," which is associated with an increased susceptibility to secondary infections, placing patients at risk for developing poor clinical outcomes (3).

Interestingly, T lymphocyte dysfunction is not unique to patients with sepsis and is observed in other illnesses, including patients undergoing elective surgery and after trauma and in patients with cancer (4). These other illnesses provide Reizine et al. (2) insight to test the hypothesis that similar mechanisms of T lymphocytes suppression occur in sepsis (5).

Arginine is considered a conditionally essential amino acid, with evidence that deficiency states can rapidly occur in different illnesses. Arginine plasma levels are maintained through a combination of protein recycling, dietary intake, and endogenous synthesis. Arginine participates as a substrate for four enzymatic processes, of which the production of nitric oxide (by three different nitric oxide synthase isoenzymes) and the generation of ornithine and urea from arginine by two arginase isoenzymes remain the best-studied. As a substrate for these enzymes, arginine deficiency may affect significant physiologic functions such as the production of endothelial nitric oxide, vasodilation and tissue oxygenation, and protein translation, resulting in cell-cycle arrest. Arginine deficiency also results in mitochondrial dysfunction.

The mechanisms that lead to arginine deficiency in different illnesses and the poor clinical outcomes that result from impaired biochemical and physiologic processes have been elucidated through the last 20 y (Fig. 1). In 2001, our laboratory reported a significant increase in arginase activity secondary to the induction of arginase 1 (ASE1) expression isolated from the mononuclear cell layer in Ficoll-Hypaque gradients in humans after trauma and in

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Competing interest statement: J.B.O.G. was the Chief Medical Officer for North America for Nestle Health Science until July 1, 2018 and does not have any current legal, contractual, or economic ties with this company or any interest in any other company. He was the Medical Director for the Surgical Intensive Care Unit at Ochsner Medical Center until November 2019. Ochsner Health and Louisiana State University jointly submitted a patent on arginine deficiency, arginase, and MDSC in COVID-19 but J.B.O.G. does not know the status of this patent.

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splenic tissues in rodents after moderate physical injury (6–8). Parallel to our research in trauma, cancer immunologists were working on a group of myeloid cells that exhibited T lymphocyte-suppressive activities. Typical scientific cross-breeding was instrumental in "putting things together," demonstrating that the suppressive myeloid cells in cancer and the newly isolated trauma-induced myeloid cells regulated T lymphocytes through a unique mechanism—that of controlling arginine availability. These groups of myeloid cells, generally immature in nature, are now called myeloid-derived suppressor cells (MDSC) (9).

It is time to move from documenting the presence of MDSC and understanding their clinical roles to discovering active and effective treatments.

But mice are not humans, and deductive, deconstructive scientific approaches are unaware of the complexity of disease in human beings. Reizine et al. (2) have importantly realized the significance of the shortcomings of deconstructive models. To that effect, they have created a more complex integrated rodent model where an initial episode of sepsis is followed by a secondary infection. Like models created in trauma (more than cancer), their model mimics a secondary infection as is observed in acutely and critically ill human beings (5, 10). In fact, our team, years ago, demonstrated that a moderate amount of physical injury similar in many ways to that reported by Reizine et al. in their sham control group is probably sufficient to explain some of the anomalies observed in their results, including a lack of alterations in mitochondrial respiration in the experimental and control groups (11). Despite the shortcomings, their model is central to understanding T cell suppression after sepsis. Thus, the effort spent is a significant contribution by Reizine et al., setting an example for other investigators.

Arginine depletion by MDSC is observed under physiologic conditions. In pregnancy, for example, arginine levels drop significantly, contributing to a state of immune tolerance, avoiding immune-mediated fetal rejection (12). However, MDSC-mediated arginine deficiency also occurs in pathologic states where characteristic clinical manifestations are also present. In 2007 our team coined the term "arginine deficiency syndrome" (ADS) to better describe the laboratory, physiologic, and clinical manifestations of arginine deficiency, which are now known to be present in several disease states including physical injury (13). Further description of ADS was done in a seminal article by Sidney Morris (14). ADS is now known to be present in certain cancers such as renal cell carcinoma, hemolytic disease (associated with the release of arginase 1 from erythrocytes and not from MDSC), trauma, and possibly some infections.

It is logical to assume that overcoming arginine deficiency in pathologic states is going to result in improved clinical outcomes. The dietary supplementation of arginine along with other frequently deficient nutrients such as omega-3 fatty acids is known as immunonutrition. More than 30 y ago, investigators observed that certain nutrients appeared to modify immune responses, improving T lymphocyte function while modulating innate inflammatory responses. These "immune-enhancing diets" were tested in heterogeneous critically ill patients and in patients undergoing elective surgery. It is only in elective surgery where consistent clinical benefit is observed, including a significant decrease in the risk of infections postoperatively and improved wound healing (15). These consistent improved clinical outcomes are associated with overall public health benefits, including a decrease in hospital length of stay and readmission rates, all contributing to lower cost of care (16). As such, the use of immunonutrition should be a standard of care, at least in certain clinical conditions that require complex surgical interventions.

Restoring arginine levels under physiologic conditions could potentially result in deleterious effects, however. Sepsis, with its paradoxical inflammatory responses associated simultaneously with T lymphocyte dysfunction, is a particularly difficult condition to treat. In fact, the use of immunonutrition has generated highly confusing results, with both reported increased and decreased mortality (17). Is it that low arginine levels in sepsis constitute a pathologic nutritional deficiency demanding treatment, or is arginine deficiency a physiologic state where MDSC are playing a modulatory role preventing an out-of-control inflammatory response, including that of an excess in nitric oxide production (18)? Only carefully conducted clinical trials will help answer this question.

Reizine et al. (2) assume that the decrease in arginine levels observed in sepsis is pathologic, stating that the lack of benefit observed with immunonutrition is due to ineffective restoration of arginine levels. Dietary glutamine is partially converted to citrulline in the brush border of the gut. Citrulline is then converted to arginine through a potentially inducible pathway during arginine deficiency states. Citrulline is not metabolized by the liver, making oral citrulline highly efficient at improving arginine availability, particularly in states of increased arginase activity (19). Not surprisingly, the use of glutamine or citrulline is associated with clinically demonstrated benefit in sickle cell disease and other hemolytic states, once again demonstrating the importance of understanding and treating ADS (20). It may therefore well be that citrulline is a better amino acid at treating arginine deficiency during sepsis.

Biomarkers aimed at better monitoring the restoration of arginine availability are sorely needed. Biomarkers mechanistically help us understand the metabolic and physiologic processes that lead to determining whether MDSC, and the subsequent decrease in arginine availability, are playing a physiologic role or are pathologic and should be treated. Within many possible biomarkers, CD3zeta expression has emerged as a prime possibility (21). So are other markers of T cell dysfunction, including that of abnormalities in mitochondrial respiration.

There are important and significant limitations to this study that need to be acknowledged, not as a sign of weakness in this article but as an opportunity for improvement. First and foremost, the assessment of T lymphocyte function, including that of T cell proliferation, is done in vitro, placing the cells in an artificial cell media which contains high concentrations of arginine, potentially altering the

reliability of the results observed. Cognizant of this, our laboratory opted for a more elaborate but reliable measure of T cell function in vivo, thus preserving the cells in an environment of arginine depletion. Using this model, we were able to demonstrate a significant recovery of intracellular arginine with the use of an arginase blocker (nor-hydroxy-L-arginine, NOR-NOHA) and restoration of T cell function. Furthermore, using a combined model of moderate trauma and subsequent infection we demonstrated improved bacterial clearance and an overall improvement in animal survival (5).

It is time to move from documenting the presence of MDSC and understanding their clinical roles to discovering active and effective treatments. There are many possibilities including, but not limited to, blocking arginase activity through pharmacologic agents or targeting the presence of MDSC through chemotherapy and immunotherapy. Dietary modification of arginine levels, however, seems straightforward and probably should be prioritized. Dietary modification of MDSC function is a promising therapy deserving well-performed clinical studies to determine its role.

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