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COMMENTARY

Arginine Cools the Inflamed Gut

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Maintenance of immunophysiological homeostasis and regulation of gut barrier function are essential for the defense of the host. Severe alterations, including infections and chronic inflammation, have been associated with increased intestinal permeability, leading to deregulation of gut function and homeostasis. To establish and reinforce this crucial balance, the intestinal metabolism plays a key role and has to be tightly regulated since in the human intestinal mucosa, the protein fractional synthesis rate is approximately 50% per day. This value is higher than that of other major metabolically active tissues, such as liver and muscle, and depends on the accessibility of the metabolic precursor pool (1). The amino acid L-arginine (L-Arg) is a central intestinal metabolite, both as a constituent of protein synthesis and as a regulatory molecule limiting intestinal alterations and maintaining immunophysiological functions (1, 2). Infection-associated L-Arg deficiency has been shown to contribute to immunopathology, and clinical trials involving L-Arg administration have shown substantial decreases in inflammation and infectious complications (3). In this issue, Chau and colleagues demonstrate that malaria-associated hypoargininemia impairs intestinal barrier function and predisposes the host to coinfection with *Salmonella*. Increasing bioavailability of L-Arg through oral supplementation ameliorates intestinal inflammation and pathology, demonstrating that pharmacological intervention at the metabolic-precursor level can be utilized to regulate mucosal immunohomeostasis (4).

METABOLISM AND CATABOLISM OF L-ARGININE

L-Arg is derived from the diet, turnover of proteins, and endogenous production through synthesis from L-citrulline (L-Cit) and successive actions of argininosuccinate synthetase (AS) and argininosuccinate lyase (AL), the third and fourth enzymes of the urea cycle. The major site of L-Arg metabolism is the liver, where L-Arg generated in the urea cycle is rapidly converted to urea and ornithine by arginases, however, with no net synthesis of L-Arg. Although synthesis of L-Arg from L-Cit can occur in many cell types, a major part of endogenous synthesis occurs via "the intestinal-renal axis," a postnatally established collaboration between epithelial cells of the small intestine and proximal tubule cells of the kidney. In adult animals, L-Cit is produced primarily by intestinal epithelial cells from NH_3, CO_2, and ornithine by carbamylphosphate synthetase I and ornithine transcarbamylase, the first two enzymes of the urea cycle, and is supplied to the kidney and probably to other tissues for synthesis of L-Arg (2, 5, 6).

L-Arg is a crucial amino acid that serves to modulate immune responses through conversion by several intracellular classes of enzymes, with isoforms of arginase and nitric oxide synthase (NOS) being the two major enzyme families exerting key immunological functions (7). However, catabolism of extracellular L-Arg requires active and regulated uptake via specific cationic amino acid transporters (CAT) or heteromeric amino acid transporters (HAT) that act as H^+-coupled symporters or antiporters (8). There are two isoforms of arginase, cytosolic arginase I (Arg1) and mitochondrial arginase II. Arg1 is abundant in liver as part of the urea cycle, and only low levels are found in extrahepatic tissue. In contrast, Arg2 is abundant mostly in extrahepatic tissues and cells such as kidney, brain, gut, and hematopoietic cells, with a main role in the production of L-ornithine (L-Orn), L-proline (L-Pro), and L-glutamate (L-Glu). In addition, three isozymes of NOS catalyze the production of NO and L-Cit from L-Arg. While neuronal NOS (nNOS or NOS1) and endothelial NOS (eNOS or NOS3) are mostly constitutively expressed, inducible NOS (iNOS or NOS2) expression is positively regulated by proinflammatory cytokines and microbial constituents activating pattern recognition molecules (7).

While, in healthy adults, the level of endogenous L-Arg synthesis is sufficiently great that L-Arg is not an essential amino acid, catabolic stress as well as dysfunction of the kidneys or small intestine can lead to hypoargininemia, a condition where levels of endogenous L-Arg may not suffice to meet metabolic demands. As such, L-Arg homeostasis is maintained primarily by modulation of L-Arg catabolism rather than L-Arg synthesis. Accordingly, L-Arg is classified as a semiessential or conditionally essential amino acid (9). Although it is known that regulation of L-Arg catabolism is influenced by (i) the expression levels of L-Arg transporters and L-Arg-converting enzymes, (ii) their cofactor availability, and (iii) substrate competition between the enzymatic systems (10), the molecular mechanisms and consequences of hypoargininemia on the host's immune response are poorly understood.

HYPOARGININEMIA CAUSES ALTERED INTESTINAL IMMUNOHOMEOSTASIS

L-Arg availability in the alimentary tract has previously been shown to play a key role in intestinal immunohomeostasis upon catabolic stress induced by inflammation and infection (7). L-Arg supplementation attenuated the degree of tissue damage in intestinal ischemia and promoted healing of intestinal mucosa (11, 12). Similarly, in a murine model of dextran sulfate sodium (DSS)-induced colitis, oral L-Arg treatment improved clinical parameters by dampening proinflammatory responses and inflammatory cell infiltration, leading to improved mucosal integrity and enhanced epithelial cell migration in an iNOS-dependent manner (13). In addition, infection by the intestinal pathogen *Citrobacter rodentium* was shown to cause a significant decrease in the serum L-Arg concentration, with associated infection-induced immunopa-

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thology being partially reversed after L-Arg supplementation (14). In this issue, Chau and colleagues demonstrate now that L-Arg availability is also critical for maintaining intestinal barrier function during malaria parasite infection, as malaria-associated hypoargininemia impairs intestinal barrier function and predisposes to coinfection with *Salmonella* (4).

It is known that during malaria parasite infection, L-Arg bioavailability drops due to enhanced destruction by host- and parasite-encoded arginases, utilization by host nitric oxide synthase (NOS), and increased scavenging of NO by cell-free hemoglobin from lysed red blood cells (RBCs) (15–17). Importantly, impaired bioavailability of L-Arg and NO has been shown to alter endothelial dysfunction in infected adults and has been suggested to be a major contributing factor to pathogenesis during infection (18). In a rodent model of experimental malaria, exogenous NO provision restored NO-mediated signaling in the brain, decreased systemic inflammation, reduced vascular leakage and hemorrhage in the brain, and provided marked protection (19). In addition, L-Arg infusion into adults infected with falciparum malaria parasites reversed infection-induced endothelial dysfunction (20), highlighting the importance of L-Arg bioavailability for the host's homeostasis during infection.

Immunopathology during malaria infection is also observed in the gastrointestinal (GI) tract. During severe *Plasmodium falciparum* infection, sequestration of parasitized RBCs and capillary blockage are prominent in intestinal villi and are associated with ischemia, malabsorption, and increased GI permeability (21–23). In addition, 50% of individuals with uncomplicated malaria have GI disturbances (24). Several features of malaria pathology suggest that the increased risk of developing bacteremia during malaria parasite and nontyphoidal *Salmonella* serotype (NTS) coinfection results from malaria-induced damage to the intestinal epithelium (25–27). Chau and colleagues demonstrate that malaria-associated hypoargininemia causes increased circulating and tissue histamine levels, mast cell activation, and ileal mastocytosis, which collectively alter intestinal epithelial integrity, predisposing the host to secondary bacterial infections (4).

REGULATION OF INTESTINAL BARRIER FUNCTION AND INFLAMMATION BY L-ARGININE

Malaria-induced hypoargininemia enhances parasite sequestration and basophil transmigration, as well as mast cell activation, which collectively result in an immunopathology that resembles allergic inflammation, leading to increased intestinal permeability (28). Although, their distinct contribution remains to be explored, it is likely that altered activities of L-Arg-catabolizing enzyme families, arginase(s), and NOS contribute to the observed immunopathology. It can be postulated that the activity of arginase through conversion of L-Arg into L-Orn enhances epithelial barrier function (29), while NOS activity confers an anti-inflammatory state through regulation of energy metabolism (30).

L-Orn produced by arginase is used by ornithine decarboxylase (ODC) to produce the polyamine putrescine, which is then converted into the polyamines spermidine and spermine by constitutively expressed spermidine and spermine synthases, respectively. Notably, polyamines are associated with mucosal protection in the GI tract and with intestinal epithelial cell migration. L-Orn can also be acted upon by ornithine aminotransferase (OAT) to produce L-proline (L-Pro), an important precursor in collagen synthesis, and is involved in wound healing and cell migration in fibroblasts and epithelial cells (10).

NOS activity has recently been shown to dampen inflammation through regulation of myeloid and lymphoid cell activation. Nitric oxide produced by iNOS in inflammatory monocytes and dendritic cells regulates inflammatory cytokine production, cell differentiation, and survival (31–33). In addition, the regulated release of NO by nonhematopoietic stromal cells controls the expansion of activated T cells (34–36), and T cell-intrinsic iNOS activity regulates polarization of antigen-specific $CD4^+$ T helper cells (37–39). Moreover, expression of iNOS by intestinal B cells is critical for immunoglobulin A class switch recombination and intestinal immunohomeostasis at steady state and upon intestinal infection (40). Future studies will have to detail the tissue-, cell-, and context-dependent role of iNOS and the molecular mechanisms that regulate nonhematopoietic, myeloid, and lymphoid immune cell function at steady state and during inflammation and infection. Modulating arginase- and NOS-mediated pathways through regulation of the bioavailability of L-Arg or its precursor L-Cit by oral supplementation provides an efficient and practical strategy to dampen intestinal inflammation and pathology, demonstrating that pharmacological intervention at the metabolic-precursor level can be utilized to regulate mucosal immunohomeostasis.

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