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The Role of Polyamines in the Regulation of Macrophage Polarization and Function

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Abstract

Naturally occurring polyamines are ubiquitously distributed and play important roles in cell development, amino acid and protein synthesis, oxidative DNA damage, proliferation, and cellular differentiation. Macrophages are essential in the innate immune response, and contribute to tissue remodeling. Naïve macrophages have two major potential fates: polarization to 1) the classical pro-inflammatory M1 defense response to bacterial pathogens and tumor cells, or 2) the alternatively-activated M2 response, induced in the presence of parasites and wounding, and also implicated in development of tumor-associated macrophages. ODC, the rate-limiting enzyme in polyamine synthesis, leads to an increase in putrescine levels, which impairs M1 gene transcription. Additionally, spermidine and spermine can regulate translation of pro-inflammatory mediators in activated macrophages. In this review, we focus on polyamines in macrophage activation patterns in the context of gastrointestinal inflammation and carcinogenesis. We seek to clarify mechanisms of innate immune regulation by polyamine metabolism and potential novel therapeutic targets.

Keywords

Polyamines; Macrophages; Polarization; Gastric cancer; Colitis

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Compliance with ethical standards. All studies from the Wilson Lab that are referenced here were conducted under proper ethical standards for either animal or human research.

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Polyamine Synthesis

Polyamines are naturally occurring, ubiquitously distributed amino acids that are synthesized from L-ornithine by ornithine decarboxylase (ODC; also known as ODC1) (Pegg and McCann 1982; Pegg 2009). Once transported into the cell by the solute carrier family 7 member 1/2 (SLC7A1/2), L-arginine is metabolized by arginase to L-ornithine and urea (Pegg and McCann 1982; Kakuda et al. 1999; Pegg 2009). Arginase is present in the cell in two isoforms; arginase 1 (ARG1) is abundantly found in liver and is involved in the urea cycle, and arginase 2 (ARG2) is found in kidney and localizes to the mitochondria. Ornithine is then converted into putrescine by ODC in the cytosol (Pegg 2006; Asim et al. 2010). Putrescine can then be converted to spermidine by spermidine synthase (SRM) and spermidine converted to spermine by spermine synthase (SMS) (Fig. 1). The conversions to spermidine and spermine require the transfer of an aminopropyl group that is donated by the decarboxylated form of S-adenosylmethionine, referred to as dcSAM (Pegg and McCann 1982; Pegg 2009). Methionine is first metabolized to SAM, which can then be decarboxylated to dcSAM by S-adenosylmethionine decarboxylase (SAMDC), which is encoded by the gene *AMD1*. The activity of SAMDC is positively regulated by putrescine and inhibited by spermidine (Pegg 2009).

Polyamines play a role in a wide range of cellular functions including cell development, amino acid and protein synthesis, oxidative DNA damage, proliferation, and differentiation (Pegg 2009). More recently, it has been found that polyamine levels may contribute to alterations of histone modifications and chromatin structure consequently affecting DNA stability and transcription (Hobbs and Gilmour 2000; Huang et al. 2009; Brooks 2013; Pasini et al. 2014; Hardbower et al. 2017a; Singh et al. 2018). The overall rate-limiting step of polyamine synthesis, the decarboxylation of L-ornithine by ODC, has been highly studied and inhibition of ODC by difluoromethylornithine (DFMO) has entered clinical trials as a treatment to prevent relapse in patients with neuroblastoma and in patients at high risk of developing colorectal or gastric adenocarcinoma (Pegg 2006; Linsalata et al. 2014; Chaturvedi et al. 2015; Saulnier Sholler et al. 2015; Bassiri et al. 2015). Endogenous regulation of ODC is achieved by the induction of antizyme, a natural inhibitor of ODC that is translationally controlled by polyamine levels (Hayashi et al. 1996). Antizyme directly binds to ODC, triggering its rapid degradation by the proteasome.

Macrophage Polarization and Function

Macrophages are bone marrow-derived monocytes and are the first line of defense against invading pathogens by acting as a surveillance system, and are thus a key component of the innate immune response. The fate of macrophages is dependent on environmental factors that stimulate polarization to either classically activated pro-inflammatory M1 or alternatively activated M2. Specific ligands mediate these distinct changes through toll-like receptors. Pathogen-associated molecular markers such as lipopolysaccharide (LPS), damage-associated molecular markers, growth factors, and IFN- γ , a Th1 cytokine, elicit pro-inflammatory activation, while Th2 cytokines, such as IL-4 and IL-13, elicit the alternative response (Anderson and Mosser 2002; Benoit et al. 2008).

Nitric oxide (NO) production, through upregulation of inducible NO synthase (NOS2), is a key characteristic of M1 macrophages (Martinez and Gordon 2014). These classically activated M1 macrophages also produce high levels of reactive oxygen species (ROS) and pro-inflammatory cytokines, including TNF α , IL-1 β , and IL-12, contributing to pathogen killing and recruitment of other pro-inflammatory cell types (Mosser and Edwards 2008). In contrast, tissue remodeling, wound healing, tumor environment regulation, allergic reactions, and responses to helminths involve M2 macrophages (Strauss-Ayali et al. 2007). These alternatively activated macrophages have enhanced arginase activity and produce IL-10. Due to the high diversity of M2 macrophage functions, the alternatively activated subset can further be subdivided into regulatory macrophages (Mregs), tumor-associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), and profibrotic macrophages (M2a) (Mosser 2003; Mosser and Edwards 2008; Murray and Wynn 2011). Each of these M2 subtypes have distinct activators and effector roles, but are overall immunosuppressive. Mregs are both activated by, and secrete IL-10, express neither arginase nor NOS2, but function to suppress the classically activated M1 macrophages (Fleming and Mosser 2011; Martinez and Gordon 2014). Tumor-derived factors within the tumor environment (e.g. hypoxia), in addition to the classic M2 stimuli, promote the differentiation and polarization of TAMs (Yang and Zhang 2017). This contributes to both the initiation and progression of tumor growth by immune suppression and angiogenesis (Yang and Zhang 2017). MDSCs are thought to be the predecessors of TAMs, but have high expression of GR1, a proinflammatory marker, in mice, low expression of F4/80, and have both arginase and NOS2 activity (Gabilovich and Nagaraj 2009). MDSCs function to suppress the innate and T-cell response in cancer. M2a macrophages express fibronectin and secrete high amounts of IL-4 and IL-13, promoting wound healing and extracellular matrix formation (Lech and Anders 2013). Once activated, macrophages retain plasticity and can switch from one functional phenotype to another based on environmental signals, but excessive activity of either polarization state can result in tissue damage, inflammatory disease, fibrosis, or tumor growth (Galli et al. 2011; Wynn et al. 2013; Lichtnekert et al. 2013; Hardbower et al. 2017b; Coburn et al. 2018; Singh et al. 2018). Thus, understanding the mechanisms of macrophage regulation is essential for disease management.

Polyamines in the context of *Helicobacter pylori* infection

Helicobacter pylori is a Gram-negative bacterium that selectively colonizes the human stomach and can cause a spectrum of disease from chronic gastritis to gastric cancer. Our lab has shown that *H. pylori* induces dysregulation of polyamine synthesis and metabolism that affects disease progression (Chaturvedi et al. 2010, 2011, 2014b, 2015). Expression of the inducible arginine transporter SLC7A2, also known as cationic amino transporter 2 (CAT2), was found to be upregulated in both mouse and human gastric tissues with *H. pylori*-induced gastritis (Kakuda et al. 1999; Chaturvedi et al. 2010). *H. pylori* infection also upregulates ARG2 and ODC expression in human gastric tissues at both the mRNA and protein levels, however, ARG1 is not induced (Gobert et al. 2002; Lewis et al. 2010). This upregulation of ARG2 comes at a cost to the host: ARG2 competes with NOS2 for the availability of L-arginine (Lewis et al. 2010). NOS2 produces NO as a defense against invading pathogens and upregulation of arginase has been shown as a survival mechanism by the intracellular

bacteria *Chlamydia psittaci*, *Chlamydia pneumoniae*, *Mycobacterium tuberculosis*, intracellular parasites, *Leishmania major* and *Toxoplasma gondii*, and the extracellular parasite *Trypanosoma brucei brucei* (Gobert et al. 2000; Iniesta et al. 2001; Huang et al. 2002; Duleu et al. 2004; El Kasmi et al. 2008). In addition, during *H. pylori* infections, the increased activity of ARG2 directly inhibits the translation of NOS2, effectively inhibiting NO production (Lewis et al. 2010).

In conjunction with upregulation of ARG2, it has been reported that *H. pylori* also induces the increased expression of spermine oxidase (SMOX), an enzyme responsible for the catabolism of spermine to spermidine (Chaturvedi et al. 2012). This induction is highly dependent on the presence of the *H. pylori* virulence factor CagA, which is also associated with a high risk of developing gastric cancer (Chaturvedi et al. 2011). Patient gastric tissue and *in vitro* studies using clinical isolates showed higher levels of SMOX expression and risk of developing gastric cancer in correlation with functional CagA secretion (Chaturvedi et al. 2011, 2015). The back-conversion of spermine to spermidine is also responsible for the release of H₂O₂, leading to DNA damage and apoptosis. Gerbil studies showed that inhibition of either ODC or SMOX reduced the rate of adenocarcinoma development and DNA damage in cells resistant to apoptosis (Chaturvedi et al. 2015).

The role of polyamines in macrophage activation and function – Stomach

Macrophages are among the first cells recruited to the gastric lamina propria and play a significant role in the pathogenicity of *H. pylori* infection. To demonstrate the involvement of macrophages in gastritis, Kaparakis et al. were able to show that mice injected with dichloromethylene diphosphonate (Cl₂MDP)-loaded liposomes had an overall depletion of circulating CD11b⁺ cells of the monocyte/macrophage lineage and substantial reduction of the recruitment of CD11b⁺ cells to gastric tissues, normally seen with *H. pylori* infection (Karakakis et al. 2008). This reduction did not affect the colonization or survival of *H. pylori*, however, there was markedly less inflammation suggesting that macrophages are a key contributor to the pathogenicity of *H. pylori*-associated gastritis (Karakakis et al. 2008). This supports one of the hallmarks of *H. pylori* infection: evasion of the elicited innate and adaptive immune response leads to the chronic inflammation responsible for the progression of infection to gastric adenocarcinoma (Wilson and Crabtree 2007; Peek Jr et al. 2010; Wroblewski et al. 2010). We propose that one mechanism underlying the ability of *H. pylori* to evade the immune response is through the dysregulation of polyamine metabolism and competition for L-arginine availability in macrophages, thus modulating both effector functions and signaling (Fig. 2).

Arginase in Macrophages

Our lab has shown that the L-arginine metabolic enzymes induced in gastric tissue and epithelial cells are also upregulated in macrophages during *H. pylori* infection. L-arginine is selectively transported into macrophages by SLC7A2 during *H. pylori* infection (Yerhamian et al. 2006; Chaturvedi et al. 2010). There is a significant increase of *Arg2*, but not *Arg1*, mRNA expression in *H. pylori*-infected murine macrophages, with the cell line RAW 264.7, and also in primary macrophages (Gobert et al. 2002; Lewis et al. 2010). Enhanced

expression of ARG2 protein levels correlates with the increase in gene expression; ARG1 protein was, again, not induced (Gobert et al. 2002; Lewis et al. 2010). Past studies demonstrate that the induction or inhibition of arginase and NOS2 inversely vary due to direct competition for available L-arginine. Peritoneal macrophages isolated from *Arg2* knockout mice infected with *H. pylori*, express increased levels of NOS2 and produce more NO compared to infected wild-type (WT) mice (Lewis et al. 2010). These findings were confirmed *in vivo* using *H. pylori*-infected mice with treatment of the arginase inhibitor *S*-(2-boronoethyl)-L-cysteine (BEC) or ARG2 deletion (Lewis et al. 2010).

ARG2 impairs the host response to *H. pylori* in addition to competing with NOS2 for the available L-arginine (Lewis et al. 2011). *Arg2* knockout mice infected with *H. pylori* have decreased bacterial colonization and increased histologic inflammation compared to infected WT mice (Lewis et al. 2011). Additionally, these mice exhibit enhanced transcription of the genes encoding the pro-inflammatory cytokines IFN- γ , IL-12p40, and IL-17A, and downregulation of the immune regulatory cytokine IL-10 (Lewis et al. 2011). ARG2 deletion also results in an increased influx of macrophages to the gastric tissues with infection, and these recruited macrophages express higher levels of NOS2 with less evidence of macrophage apoptosis (Lewis et al. 2011). A more recent study from our laboratory has expanded on these findings and demonstrates the effects of ARG2 on macrophage polarization (Hardbower et al. 2016). Bacterial colonization and gastric inflammation do not differ between *Arg2* knockout and *Arg2:Nos2* double knockout mice, suggesting that the effects of ARG2 *in vivo* are independent of NOS2 (Hardbower et al. 2016). *Arg2* knockout mice exhibit enhanced M1 macrophage activation through increased mRNA expression of *Irfg*, *Il17a*, *Nos2*, *Il1b*, and *Tnfa*, and increased production of the pro-inflammatory markers TNF- α , IL-1 β , CCL3, CCL4, CCL5, and NO in both gastric tissues and bone marrow-derived macrophages (BMDMs) (Hardbower et al. 2016). Loss of ARG2 also modulates changes in the expression of enzymes involved in the polyamine metabolism pathways. The genes encoding ARG1, ODC, SAMDC, diamine acetyltransferase 1 (SAT1), and SMOX are all upregulated in *H. pylori*-infected gastric tissues and BMDMs as a compensatory mechanism for ARG2 deficiency (Hardbower et al. 2016). The decrease of putrescine and increase of spermine by ARG2 deficiency also elicited a more vigorous Th1/Th17 response, indicating that the expression of Th1/Th17 cytokines in the *H. pylori*-infected stomach is dependent on relative polyamine levels (Hardbower et al. 2016). Altogether, these data support the concept that metabolism of L-arginine by arginase drives macrophages away from an M1 response.

To assess the role of chronic inflammation on macrophage function, Chaturvedi et al. isolated gastric macrophages after a 4 month infection with *H. pylori* and re-stimulated them with *H. pylori* lysate (Chaturvedi et al. 2010). These cells exhibited increased expression of *Slc7a2*, *Odc*, and *Nos2* (Chaturvedi et al. 2010). Nevertheless, the increase of mRNA did not result in a corresponding increase in protein levels in macrophages isolated from the infected mice. In fact, there was a decrease in NOS2 protein levels, NO production, and L-arginine uptake compared to cells from uninfected mice, suggesting that polyamine synthesis during chronic infection may favor and maintain an M2-like response, in that M1 responses are blunted (Chaturvedi et al. 2010). Moreover, in that study it was shown that spermine can impair L-arginine uptake into macrophages, providing one potential mechanism for

diminished NOS2 protein expression and NO production (Chaturvedi et al. 2010) as *H. pylori*-stimulated NOS2 protein translation is specifically dependent on L-arginine availability in macrophages (Chaturvedi et al. 2007).

ODC and SMOX in Macrophages

Not only does *H. pylori* induce ARG2, but infection upregulates the expression of ODC in macrophages (Gobert et al. 2002). Similar to ARG2, the induction of ODC diverts the utilization of L-arginine towards polyamine synthesis and away from NOS2. Bussière et al. knocked down *Odc* expression with siRNA, demonstrating an inverse relationship with NOS2 (Bussière et al. 2005). *Odc* knockdown increased NOS2 protein expression and NO production, without affecting *Nos2* mRNA levels, which was associated with decreased spermine concentrations within macrophages (Bussière et al. 2005; Chaturvedi et al. 2010). Inhibiting ODC and putrescine synthesis in *H. pylori*-infected BMDMs with the pharmacological inhibitor DFMO decreased levels of putrescine, supporting the host immune response by enhancing the M1 phenotype without altering M2 activation (Hardbower et al. 2017a). These findings are recapitulated *in vivo* with mice treated with DFMO and inoculated with *H. pylori* (Chaturvedi et al. 2010; Hardbower et al. 2017a). There is an increase of arginine uptake, *Nos2* translation, and NO production in gastric macrophages. The outcome of this upregulation is decreased *H. pylori* colonization and decreased gastric inflammation in infected mice (Chaturvedi et al. 2010; Hardbower et al. 2017a). Supplementation of putrescine in BMDMs rescues ODC inhibition by decreasing M1 macrophage and NLR family, pyrin domain containing (NLRP) 3-driven inflammasome activation demonstrating that putrescine, generated by the induction of ODC, has a role in macrophage function (Hardbower et al. 2017a).

In partial contrast to the findings with DFMO, myeloid specific *Odc* knockout (*Odc^{mye}*) mice exhibit decreased *H. pylori* colonization, but increased histological inflammation scores (Hardbower et al. 2017a). Using *Odc^{mye}* mice, Hardbower et al. demonstrated that ODC and putrescine alter histone modifications and attenuate the M1 response (Hardbower et al. 2017a). H3K4 monomethylation (H3K4me1) and H3K9 acetylation (H3K9ac) are known histone modifications that enhance euchromatin formation and thus gene expression, while H3K9 di/trimethylation (H3K9me2/3) is associated with decreased gene expression (Georgopoulos 2002; Shlyueva et al. 2014). Hardbower et al. found that during *H. pylori* infection of *Odc^{mye}* BMDM, there is a significant increase of H3K9me1 and H3K9ac, but a decrease of H3K9me2/3 (Hardbower et al. 2017a). Treatment with BIX 01924, a selective inhibitor of H3K9 methyltransferase, removed the inhibitory methylation of *Odc^{fl/fl}* BMDMs and increased M1 markers, while having no effect on *Odc^{mye}* BMDMs (Hardbower et al. 2017a). In contrast, treatment with anacardic acid, an inhibitor of lysine transferase 2A, reversed the increased M1 expression in *Odc^{mye}* BMDMs while having no effect in *Odc^{fl/fl}* BMDMs (Hardbower et al. 2017a). The histone modification and euchromatin formation of *H. pylori*-infected *Odc^{mye}* mice leads to an increase of gene expression of macrophage-derived proinflammatory markers *Il1b*, *Il6*, *Il12a*, *Il12b*, *Tnfa*, and *Nos2* in gastric tissue and stimulated BMDMs (Hardbower et al. 2017a). Infection of *Odc^{mye}* mice resulted in an increase in production of proinflammatory cytokines CCL2, CCL3, CCL4, CCL5, CXCL1, CXCL2, CXCL10, IL-17, and TNF- α (Hardbower et al.

2017a). This enhanced M1 macrophage activation was also observed in human THP-1 macrophage-like cells treated with DFMO (Hardbower et al. 2017a). The exogenous addition of putrescine reversed the histone modifications and M1 marker expression observed in *Odc^{mye}* BMDMs, highlighting the role of putrescine in macrophage polarization (Hardbower et al. 2017a) (Fig. 1).

Increased polyamine synthesis provides SMOX with available substrate. Since the catabolism of spermine to spermidine by SMOX produces H₂O₂, this upregulation results in increased macrophage apoptosis of *H. pylori*-infected RAW 246.7 cells (Chaturvedi et al. 2004). Chemical inhibition of SMOX or detoxification of H₂O₂ with catalase attenuated the infection-induced apoptosis (Chaturvedi et al. 2004). Our lab also found that knocking down SMOX expression with shRNA decreased arginine uptake by RAW 246.7 macrophages (Chaturvedi et al. 2014a). This resulted in a decrease of *H. pylori* lysate-stimulated production of NO and an increase in *H. pylori* survival (Chaturvedi et al. 2014a). These results were reversed with transfection of a SMOX overexpression vector with subsequent decrease of spermine levels in RAW 246.7 cells and the human monocyte cell line THP-1 (Chaturvedi et al. 2014a). Bussière et al. found that increased concentrations of spermine within the cell correlated with decreased NOS2, attributed to a direct inhibition of spermine on the translation of NOS2 (Bussière et al. 2005). In combination, these findings suggest that the macrophage effector function of pathogen killing by NO production is either downregulated by spermine or upregulated by spermidine (Fig. 2).

The role of polyamines in macrophage activation and function – Colon

Polyamine regulation of macrophage polarization is not specific to *H. pylori*-induced inflammation within the gastrointestinal tract. ODC and arginase are upregulated during infection with the colonic pathogen *Citrobacter rodentium*, increasing polyamines and decreasing NO production (Gobert et al. 2004). In contrast to *H. pylori*-induced gastric inflammation, ARG1 is upregulated during *C. rodentium* infection and other mouse models of colitis (Gobert et al. 2004). Knocking out NOS2, even with L-arginine supplementation, reduced markers of M1 activation (Gobert et al. 2004). Arginine supplementation did, however, increase the total concentration of polyamines (Gobert et al. 2004). *Odc^{mye}* macrophages infected with *C. rodentium* displayed increased H3K9ac and decreased H3K9me2/3 levels indicative of euchromatin and enhanced capacity for gene transcription, and accordingly, infected *Odc^{mye}* mice exhibit significantly increased histologic colitis and disease severity (Hardbower et al. 2017a).

Inflammatory bowel disease (IBD) is a strong risk factor for developing colitis-associated carcinogenesis (CAC) because of the constant inflammatory state (Ekbom et al. 1990; Eaden et al. 2001). Macrophage ODC augments epithelial injury-associated colitis and CAC by impairing the M1 responses that can stimulate epithelial repair, antimicrobial defense, and antitumoral immunity (Singh et al. 2018). As with *H. pylori* infections, myeloid cell-specific deletion of *Odc* results in upregulation of the M1 response during induced colonic inflammation (Singh et al. 2018). ODC-expressing macrophages are also significantly increased in the colonic tissue of patients with Crohn's disease, active ulcerative colitis, dysplasia, and colitis-associated colon cancer compared to normal or inactive ulcerative

colitis tissues (Singh et al. 2018). Singh et al. used dextran sulfate sodium (DSS) and azoxymethane (AOM)-DSS as models of colitis and CAC, respectively. *Odc^{mye}* mice treated with DSS exhibited lower histologic injury scores (Singh et al. 2018). *Odc^{mye}* mice treated with DSS also had a significant increase of the pro-inflammatory cytokines CSF2, TNF α , IL-1 α , and IL-1 β ; the prototype Th1 cytokine IFN γ ; and the chemokines CXCL2, CXCL9, CXCL10, CCL2, CCL3, CCL4, CCL4, and CCL5; and a significant increase in the mRNA expression of the M1 genes *Nos2* and *I11b*, and no change in the M2 genes *Arg1* and *Chil3* (Singh et al. 2018). When assessing disease progression, *Odc^{mye}* mice treated with AOM-DSS exhibited a significant decrease in tumor number, tumor burden, and severity of dysplasia (Singh et al. 2018). There were also notable differences in the tumor versus non-tumor tissues in the AOM-DSS model (Singh et al. 2018). In tumor tissues of the *Odc^{mye}* mice that received AOM-DSS, there was a significant increase of the M1 markers *Nos2*, *I11b*, *I112a*, *I112b*, *Tnfa*, and the Th1 marker *Ifng* (Singh et al. 2018). The protein levels of NOS2, TNF- α and IFN- γ were also significantly increased (Singh et al. 2018). These findings provide evidence that inhibiting ODC may be protective against CAC due to the enhanced pro-inflammatory/anti-tumoral activity of M1 macrophages activated by lowered levels of putrescine and the resulting increase of histone modifications and euchromatin formation, which was demonstrated by increased macrophage immunofluorescent staining for H3K9ac in colon tumor macrophages of *Odc^{mye}* mice (Singh et al. 2018).

AB-hydrolase containing 5 (ABHD5) is a co-activator of the adipose triglyceride lipase (ATGL), a key enzyme involved in lipolysis of triglycerides into diglycerides and free fatty acids (Lass et al. 2006). ABHD5 expression is shown to be increased in TAMs during colorectal cancer (CRC) (Miao et al. 2016). TAMs are rich in lipid droplets, thus ABHD5 may be a key factor in TAM utilization of the lipid deposits for energy (Miao et al. 2016). The anti-tumor response via M1 macrophages is suppressed by the enhanced TAM survival in the tumor environment of CRC. The M2 response is also sustained by ABHD5-mediated suppression of SRM and accumulation of putrescine (Miao et al. 2016). ABHD5 suppresses SRM in macrophages by inhibiting the reactive oxygen species-dependent expression of C/EBP ϵ , a transcription factor of the gene encoding SRM (Miao et al. 2016). Overexpression of ABHD5 in macrophages supported tumor growth while knocking down ABHD5 suppressed tumor growth by increasing spermidine production through C/EBP ϵ (Miao et al. 2016). Spermidine, by overexpression of SRM is also able to directly inhibit the growth of CRC cells (Miao et al. 2016). Tumor progression was rescued by knocking down C/EBP ϵ and inhibiting *Srm* transcription (Miao et al. 2016). ABHD5 suppression of spermidine synthesis is another example of the how polyamines can be instrumental in the balance between M1 versus M2 macrophage activation (Fig. 2).

The role of polyamines in macrophage activation and function – Other

Tumors

In a study to characterize the effects of novel polyamine blockade treatment (PBT), Alexander et al. demonstrated that co-treatment with DFMO and a trimer polyamine transport inhibitor (PTI) decreased the M2 response and increased the anti-tumor M1 immune response (Alexander et al. 2017). The PBT inhibits polyamine synthesis as well as

exogenous polyamine uptake, thus depleting polyamine concentrations (Alexander et al. 2017). The treatment promoted macrophage infiltration of various tumors *in vivo* marked by increased concentrations of IL-10, IFN- γ , and monocyte chemoattractant protein-1 (MCP-1) (Alexander et al. 2017). The alteration of cytokine production decreased immunosuppressive activity of MDSCs and increased cytotoxic T cell activity (Alexander et al. 2017).

Bacterial-induced Inflammation

All bacterial pathogens elicit an innate response upon detection at the site of infection. RAW 264.7 cells and BMDM treated with various inflammation-inducing stimuli including LPS, *Bacillus Calmette–Guerin* (BCG, TB vaccine), and carbon tetrachloride (CCl₄, hepatotoxin), produce varying responses to modulations of ODC (Jiang et al. 2018). Knockdown of ODC resulted in increased TNF- α , IL-1 β , and IL-6 when stimulated with LPS, and decreased IL-1 β and IL-6 when stimulated with BCG and CCl₄ (Jiang et al. 2018). Overexpression of *Odc* resulted in decreased TNF- α , IL-1 β , and IL-6 when stimulated with LPS, and an opposite effect with BCG or CCl₄ (Jiang et al. 2018). This suggests that macrophages respond differently to varying stimuli and highlights the need to screen the response of ODC to small molecules and the resulting effect on immune cell function. Inhibition of ODC potentiates NO production while spermine supplementation inhibits production of NO in LPS-activated murine macrophage J774 cells (Szabó et al. 1994; Baydoun and Morgan 1998). Increased concentrations of DFMO correlated with increased concentrations of NO with LPS stimulation in the first 48 hours of LPS stimulation (Baydoun and Morgan 1998). Spermine post-translationally suppresses the production of IL-12p40 in peritoneal macrophages, coinciding with decreased INF- γ (Hasko et al. 2000). Spermine also inhibits proinflammatory cytokine synthesis in human mononuclear cells, a counterregulatory mechanism that restrains the immune response (Zhang et al. 1997). Increased concentrations of spermine pre-treatment decreased TNF, MIP-1 α , MIP-1 β , IL-1 β , IL-6, and TGF- β 1 secretion of human peripheral blood mononuclear cells (PBMCs) stimulated with LPS (Zhang et al. 1997). TNF secretion was also inhibited in RAW 264.7 cells with LPS stimulation (Zhang et al. 1997). Alveolar infection by *Pneumocystis* organisms reduces levels of antizyme inhibitor (AZI), an endogenous inhibitor of the ODC inhibitor, antizyme, resulting in more active ODC combined with an increase in polyamines. This further increases overall polyamine levels within macrophages favoring *Pneumocystis* survival via an M2 response and macrophage apoptosis (Liao et al. 2009).

Liver Inflammation

Spermine pre-treatment of Kupffer cells (resident liver macrophages) isolated from mice injected with thioacetamide, a hepatotoxin that causes acute and chronic liver injury, had reduced expression of *Illb* and *Nos2*, but increased expression of *Arg1* and *Mrc1* (macrophage mannose receptor 1) (Zhou et al. 2018). The spermine pre-treatment also resulted in a decrease of CCL2, CXCL-10, TNF- α and IL-6, but an increase of IL-10 in the tissues, thus suppressing the recruitment of immune cells (Zhou et al. 2018). This is supported by low levels of proinflammatory markers and increased IL-10 in the serum, and evidence of less macrophage infiltration in the tissues (Zhou et al. 2018). Knockdown of autophagy protein 5 (ATG5) mediates autophagy and increases M1 markers, while decreasing M2 markers (Zhou et al. 2018) (Fig. 2). This suggests that the Kupffer cell

M1/M2 polarization is autophagy-dependent and spermine enhances autophagy via increased ATG5 expression. Overall, spermine attenuates injury and apoptosis due to thioacetamide in the liver.

Parasites

Upregulation of L-arginine transport during *Leishmania donovani* infection leads to increased levels of polyamines and an M2 response characterized by increased IL-10 and decreased IL-12 and TNF- α (Mandal et al. 2017). *Leishmania donovani* is an intercellular parasite and can reside in macrophages; this survival is L-arginine dependent and SLC7A2 is needed for arginine transport (Mandal et al. 2017). *Leishmania donovani* promotes arginine uptake upregulating ARG2 and ARG1 expression, however, there is no observed change in the expression of NOS2 (Mandal et al. 2017).

Allergy/Asthma

High levels of circulating polyamines have been reported in the blood of patients with severe asthma (Jain 2018). M2 macrophages were among the most prevalent immune cells within the lung of asthmatic patients due to a Th2-rich environment (Jain 2018). A characteristic of macrophages activated in response to asthma and *Taenia crassiceps* helminths is the expression of E-cadherin. Van den Bossche et al. found that the interaction of E-cadherin with catenins is regulated in a polyamine-dependent manner (Van den Bossche et al. 2009). M2 macrophage activation by IL-4 requires polyamines, more specifically putrescine, to have fully functional E-cadherin and acquire the ability to adhere to other cells (Van den Bossche et al. 2009). Dysregulation of polyamine synthesis by DFMO or diethylnorspermine (DENSPM), a polyamine analogue that depletes the polyamine pool, decreased *Cdh1* mRNA levels, the gene that encodes cadherin 1 (E-cadherin) (Van den Bossche et al. 2009).

Polyamine depletion resulted in increased expression of LPS-induced proinflammatory genes, but not excretion (Van den Bossche et al. 2012). Polyamine synthesis may be ARG1-independent in macrophages, as *Arg1*-deficient macrophages did not have reduced IL-4-induced polyamine production (Van den Bossche et al. 2012). Using DFMO and DENSPM, Van den Bossche et al. were able to identify multiple markers of M2 macrophages that are dependent on polyamines for IL-4-induced expression, including *Retnla*, *Chil3*, *Pdcd11g2*, *Slc7a2*, *Rnase2a*, *Ccl17*, *Cldn11*, *Cdh1*, and *Mrc1* (Van den Bossche et al. 2012).

Conclusions

Polyamines are essential for multiple processes, many of which are not fully understood and vary between beneficial and harmful. Competition for L-arginine and the relative ratios of putrescine, spermidine, and spermine contribute to the effects of polyamine metabolism on cellular function. We propose that polyamines regulate the activation and thus the role of macrophages in the innate immune response to gastrointestinal pathogens. Our lab has shown that inhibiting the production of putrescine supports innate immunity by inducing polarization of macrophages to a pro-inflammatory response. This is accomplished by either directly inhibiting ODC with DFMO, or by alleviating the competition for L-arginine by

inhibiting arginase. It has been shown that pathogens, including *H. pylori* and *L. donovani*, upregulate polyamine metabolism as a survival mechanism to impair M1 macrophage responses and produce an M2-like state and increase the rate of ROS-induced macrophage apoptosis, enhancing the risk of disease progression. These findings suggest that polyamines regulate myeloid-derived macrophage function via both biochemical pathways and epigenetic modifications providing insight into potential novel therapeutic targets. Studies, such as those ongoing in our laboratory, are needed to elucidate further the mechanisms by which polyamines alter macrophage immunometabolism, a key aspect to host cell function in the setting of infections or tumorigenesis.

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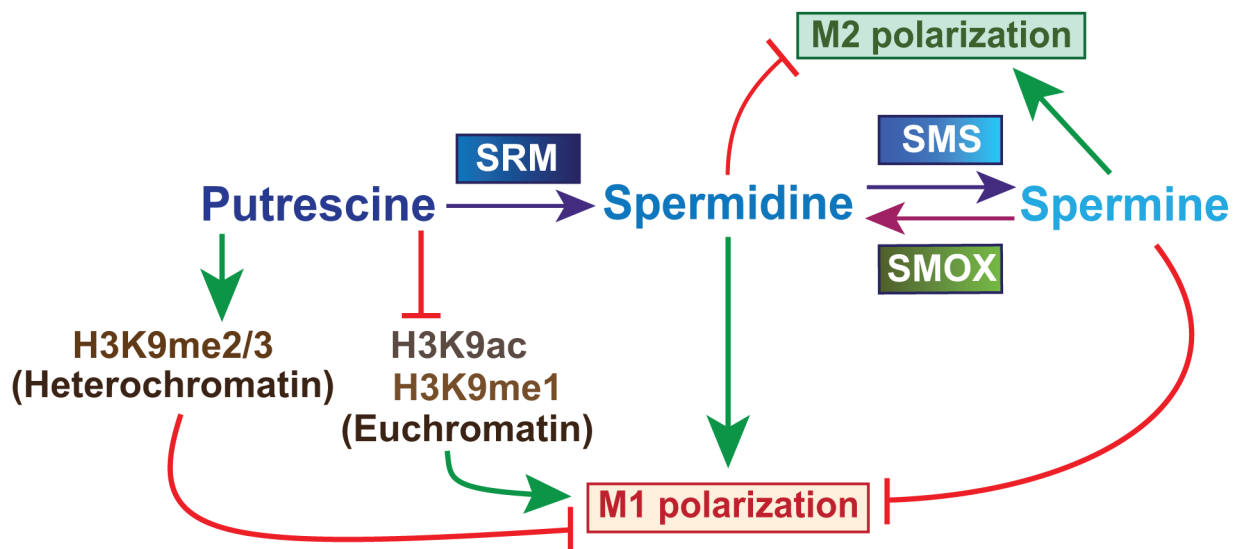


Fig. 1. Overview of polyamine synthesis and the effect on macrophage polarization. Putrescine is converted to spermidine by spermidine synthase (SRM) and spermidine is converted to spermine by spermine synthase (SMS). Spermine oxidase (SMOX) back-converts spermine to spermidine. Macrophages have two main polarization states; classically activated pro-inflammatory M1 macrophages and alternatively activated, anti-inflammatory and protumoral M2 macrophages. Putrescine inhibits the formation of euchromatin thus downregulating the expression of M1 genes. Spermidine favors M1 polarization while spermine favors M2

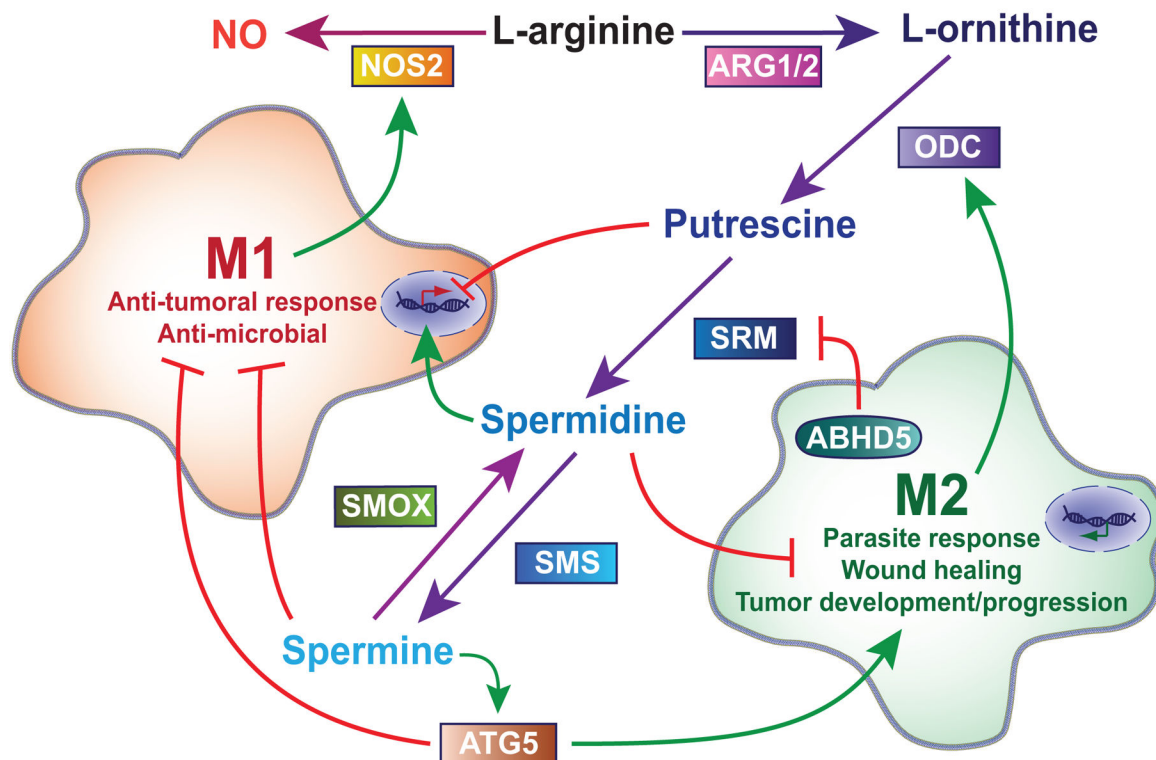


Fig. 2. Regulation of macrophage function by polyamine synthesis. In general, once activated, M1 macrophages metabolize L-arginine by NOS2 to produce NO while M2 macrophages (Mregs, TAMs, MDSCs, M2a) upregulate the ARG1/2-ODC pathway to produce polyamines, however, both retain the ability to change activation states. Putrescine downregulates transcription of M1 genes including *Nos2* and spermine inhibits the translation of NOS2, thus hindering an M1 response. Spermine also supports autophagy via ATG5, which inhibits M1 polarization while promoting M2 polarization. In contrast, spermidine upregulates transcription of *Nos2* and can both directly and indirectly inhibit tumor growth. TAM survival is mediated by upregulation of ABHD5, which suppresses spermidine by inhibiting translation of spermidine synthase (SRM)