

# Spermine Inhibition of Monocyte Activation and Inflammation

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## Abstract

The innate immune system functions as a defensive front line against pathogenic invasion, but the proinflammatory products of activated monocytes and macrophages (e.g., TNF and NO) can also injure normal cells. Anti-inflammatory mediators restrain the innate immune response and prevent excessive collateral tissue damage. Spermine, a ubiquitous biogenic polyamine, specifically and reversibly suppresses the synthesis of monocyte proinflammatory cytokines. This may provide a counterregulatory mechanism to restrain monocyte activation in injured or infected tissues and in tumors where spermine levels are significantly increased. Here we show that monocyte spermine uptake was signifi-

cantly increased following lipopolysaccharide stimulation. The polyamine analogue 1,4-bis(3-aminopropyl)-piperazine (BAP) inhibited LPS-stimulated monocyte spermine uptake via the "nonselective" polyamine transporter. BAP fully restored macrophage TNF synthesis despite the presence of spermine, indicating that the mechanism of monocyte deactivation by spermine is dependent on spermine uptake. Administration of BAP in vivo significantly augmented the development of carrageenan-induced paw edema and nitric oxide release. Thus, endogenous spermine normally inhibits the innate inflammatory response by restraining macrophages.

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## Introduction

Monocytes and macrophages occupy a critical role in the orchestration of a competent innate immune response. When activated by pathogenic stimuli or injury, macrophages release tumor necrosis factor (TNF), other cytokines, and nitric oxide (NO) that bolster defense against invasion. NO, for instance, is cytotoxic to invading organisms, and increases regional blood flow to the affected tissues (1), while TNF and interleukin-1 (IL-1) activate endothelial cells, recruit

leukocytes, enhance procoagulant activity, and facilitate tissue remodeling (2,3). The importance of these responses is revealed by studies of TNF receptor knock-out mice, which are exquisitely sensitive to infection by intracellular pathogens, reflecting the normal role of TNF to enhance host defenses against infection (4). These beneficial effects, however, are critically dependent on the magnitude of the immune response, because larger amounts of host-derived cytotoxins cause collateral damage to normal tissues (3,5,6). Moreover, when release is sufficient to cause systemic exposure, macrophage-derived cytotoxins mediate lethal shock and tissue injury (7). To prevent these potentially lethal effects, the immune system has evolved a series of counter-

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regulatory mechanisms that inhibit macrophage activation. For example, anti-inflammatory cytokines (IL-10) and TGF- $\beta$ ) and glucocorticoid hormones are released during inflammation and inhibit macrophage release of TNF and NO (8–13). Ultimately, therefore, successful resolution of pathogenic invasion depends on the beneficial activities of macrophage products within the context of a closely regulated macrophage response.

Spermine, a ubiquitous biogenic polyamine, is one of the most abundant molecules in eukaryotes. Proliferating cells and tissues generally have higher levels of polyamines, and injured cells release spermine into the extracellular milieu. Spermine levels increase significantly at sites of infection and injury (14) as well as in tumors (15). More than 40 years ago, Hirsch and Dubois discovered that local accumulation of spermine in infected tissues can curb the growth of mycobacteria (16). More recently, we found that spermine is a specific and reversible inhibitor of monocyte activation that suppresses the synthesis of TNF and other proinflammatory cytokines by human peripheral blood mononuclear cells. Spermine suppresses proinflammatory cytokine synthesis via a post-transcriptional mechanism (17). Direct application of spermine together with carrageenan, an activator of the innate immune response, attenuates the development of carrageenan-induced inflammation, suggesting that macrophages are sensitive to spermine inhibition *in vivo*. In agreement with this mechanism, macrophages derived from polyamine-depleted mice (after treatment with a polyamine biosynthesis inhibitor, DMFMO<sub>me</sub>) release more TNF and IL-1 in response to lipopolysaccharide (LPS) challenge (18). On the basis of these and other results, we reasoned that the macrophage deactivating activity of spermine might be a critical mechanism to restrain macrophages from causing excessive collateral damage. Increased spermine levels at sites of tissue injury would “inform” macrophages that extensive tissue injury had occurred, and curb their further inflammatory activity.

Polyamine transport systems in mammalian cells have been divided functionally into two classes that are distinguished by differential affinity for terminal aminobutyl or aminopropyl groups (19). One transporter class, termed “non-selective,” facilitates the uptake of spermine, spermidine, and putrescine; the other class is specific for polyamines with aminopropyl groups (spermine and spermidine) but has a low affinity

for putrescine. Cells alter the activity of the polyamine uptake systems to regulate intracellular polyamine availability (20). Whereas active polyamine transport has been demonstrated in macrophages (21), a role for macrophage polyamine transport activity in modulating inflammatory responses has not been described previously.

We now report that human monocytes express a nonselective polyamine transporter, and that LPS stimulation significantly increased monocyte uptake of spermine via this transporter. When spermine accumulation via this transport system was inhibited by treatment with the polyamine analogue 1,4-bis(3-aminopropyl)-piperazine (BAP), monocytes were not deactivated, and their proinflammatory activity was unrestrained despite the presence of elevated extracellular spermine. Application of BAP with carrageenan *in vivo* significantly increased carrageenan-induced local inflammatory responses, indicating that monocytes incorporate endogenous spermine as a normal counterregulatory mechanism to restrain the magnitude of the innate immune response.

## Materials and Methods

### *Cell Isolation and Culture*

Human leukocyte buffy coats were obtained by leukopheresis of blood from normal individual donors to the Long Island Blood Service (Melville, NY). Human peripheral blood mononuclear cells (PBMCs) were freshly isolated from 30 ml of leukocyte buffy coats by density gradient centrifugation through Ficoll-Paque using a procedure recommended by the vendor (Pharmacia, Uppsala, Sweden), with a yield typically of  $2\text{--}4 \times 10^8$  cells. Cells were resuspended in RPMI 1640 medium (Gibco BRL, Grand Island, NY) containing 10% heat-inactivated human serum, 0.1% L-glutamine, and 0.01% gentamicin, and plated in 96-well plates at  $5 \times 10^5$  cells per well in 200  $\mu$ l, then cultured at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in air. Nonadherent cells were removed after overnight culture, and adherent cells (monocyte cultures) were then used in the experiments.

### *Polyamine Uptake*

Human monocytes in 96-well plates were stimulated by adding 135  $\mu$ l of medium with 100 ng/ml freshly sonicated *Escherichia coli* endotoxin (LPS, Sigma, St. Louis, MO). After 2 to 24 hr

stimulation, 15  $\mu\text{l}$  of medium with  $^{14}\text{C}$ -labeled polyamine (Amersham, Arlington Heights, IL) was added to the final concentrations indicated in the absence or presence of BAP (Aldrich, Milwaukee, WI). Following a 25-min incubation at 4°C (for membrane binding) or 37°C (for both membrane binding and cellular uptake), media were aspirated, and the cells were rinsed three times with 200  $\mu\text{l}$  of phosphate-buffered saline (PBS). Cells were then solubilized in 100  $\mu\text{l}$  of 1 N NaOH, and 80  $\mu\text{l}$  of sample was transferred to a 96-well Lumaplate™ (Packard, Downers Grove, IL). Samples were dried at room temperature overnight, and the cell-associated radioactivity was determined on a Packard liquid scintillation counter. Polyamine uptake is defined as the difference between cell-associated radioactivity at 37°C and that at 4°C.

#### *Cytokine Assay*

To stimulate cytokine production from human monocytes, freshly sonicated LPS was added to a final concentration of 100 ng/ml. To evaluate the effect of BAP on the inhibitory activity of spermine on monocyte TNF synthesis, human monocytes were preincubated with the concentration of BAP indicated for 30–60 min and 35  $\mu\text{M}$  of spermine (Sigma) for another 30–60 min, then stimulated with 100 ng/ml of LPS. After 4 hr of LPS stimulation, supernatants were harvested for cytokine assay. TNF levels in the supernatants were determined by ELISA. Briefly, serial dilution of recombinant human TNF was used to generate standard curves. The 96-well microtiter plates coated with monoclonal anti-TNF antibodies were incubated with 60  $\mu\text{l}$  of standards or supernatant samples for 2 hr at room temperature or overnight at 4°C. After washing with buffer containing 20 mM Tris-HCl, pH 7.4, 150 mM NaCl, and 0.05% Tween-20, 60  $\mu\text{l}$  of polyclonal anti-TNF antibodies diluted (1:200) in dilution buffer (10 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.2% Tween-20, and 1% goat serum) was added and incubated at room temperature for 2 hr. Following several washes to remove the unbound antibodies, the bound antibodies were reacted for 30 min with alkaline phosphatase-conjugated goat anti-rabbit IgG (H+L) (Boehringer Mannheim, Indianapolis, IN) diluted 1:2500 in dilution buffer. After washing, the amounts of bound second antibodies were then determined by assaying for alkaline phosphatase activity with 100  $\mu\text{l}$  of freshly prepared p-nitrophenylphosphate in diethanolamine buffer (10 mM

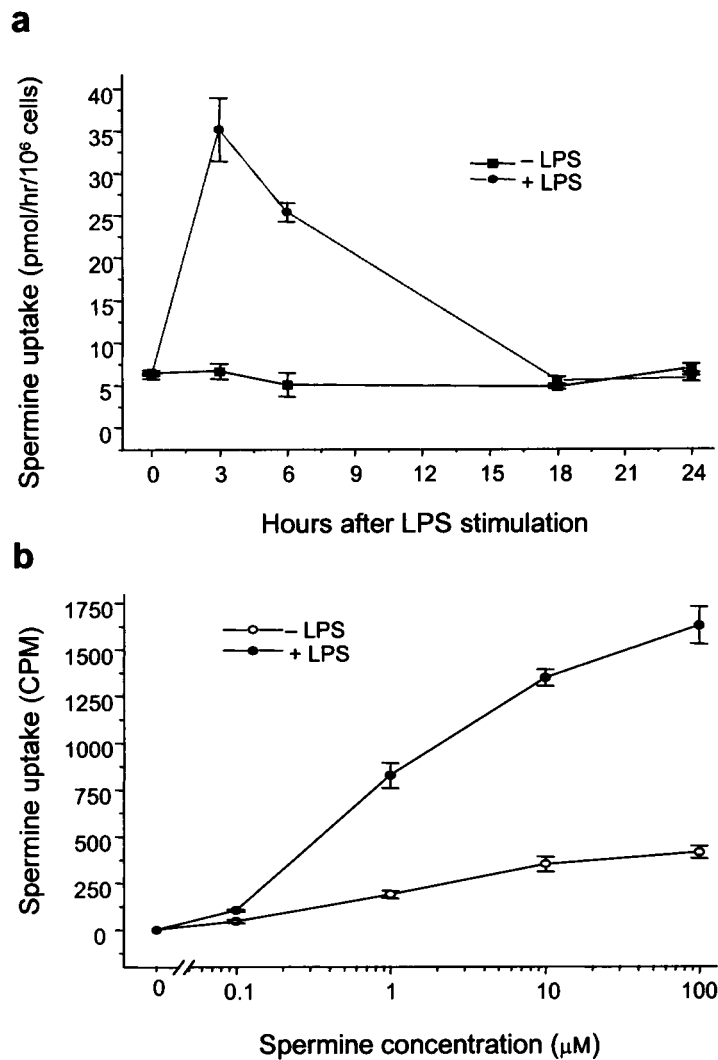
diethanolamine and 0.5 mM MgCl, pH 9.5). After 30 min incubation at room temperature, the absorption at 405 nm was determined using a microtitre plate reader for TNF levels.

#### *Carrageenan-Induced Footpad Inflammation*

Paw edema was induced by injecting 100  $\mu\text{l}$  of 0.2%  $\lambda$ -carrageenan (Sigma) in PBS, pH 7.4, into the plantar surface of the left hindpaw of male Lewis rats (300–350 g). Spermine and/or BAP at the concentrations indicated were coadministered with carrageenan. The right hindpaw was injected with 100  $\mu\text{l}$  of PBS as a control. The thickness of the carrageenan-treated and PBS-treated hindpaw was measured with calipers at the time points indicated, and the difference was used as an index of inflammation. To recover edema fluid from rat paws, rats were sacrificed 16–24 hr after carrageenan administration. Paws were excised at the level of the calcaneus bone, and placed in a 6-ml Falcon 2063 tube (a 4- to 4.5-mm hole was made with the cut side down). The 6-ml Falcon tube was then placed in a 15-ml Falcon 2097 conical tube with 50  $\mu\text{l}$  of PBS and centrifuged at 250–300  $\times g$  for 20 min and the resultant fluid was recovered for assay as described.

#### *Determination of NO Production in Rat Paw*

NO production in the edema fluid from each paw was determined by measuring nitrite/nitrate level with a nonenzymatic NO assay kit (OXIS, Portland, OR). Serial dilution of nitrite was used to generate standard curves. Briefly, 40  $\mu\text{l}$  of sample was adjusted to 190  $\mu\text{l}$  with water, and then 10  $\mu\text{l}$  of 30% ZnSO<sub>4</sub> was added for protein precipitation. After 15 min incubation at room temperature, the samples were centrifuged at 4000 rpm for 5 min, and the supernatants as well as serially diluted NO standards were transferred to microcentrifuge tubes containing 0.5 g cadmium beads (sequentially prewashed with H<sub>2</sub>O, 0.1 M HCl, and 0.1 M NH<sub>4</sub>OH, pH 9.6) and incubated at room temperature overnight with agitation to convert nitrate to nitrite. After 5 min centrifugation at 4000 rpm, 60  $\mu\text{l}$  of supernatant was added to wells in duplicate in a 96-well plate and adjusted to 100  $\mu\text{l}$  with water. Greiss reagent (100  $\mu\text{l}$ ) was then added to each well and incubated for 5 min at room temperature. The nitrite level in each edema fluid was determined by reading the absorbance at 570 nm in a microtiter plate reader, and the total NO level of each paw



**Fig. 1. Spermine uptake in human monocytes is increased by LPS stimulation.** Human monocyte cultures were treated with LPS (100 ng/ml) or vehicle PBS for 0 to 24 hr (a) or for 2 hr (b), then incubated with the indicated concentration of spermine with <sup>14</sup>C-labeled spermine tracer at either 4°C or 37°C for 25 min. Cell-associated radioactivity (CPM) was measured on a Packard microplate scintillation counter, and spermine uptake (CPM) is expressed as the difference between cell-associated radioactivity at 37°C and that at 4°C at each spermine concentration. Results are the mean of spermine uptake ± SE.

was calculated as  $X$  (nmol/paw) = nitrite/nitrate in the sample (nmol/ml) × paw edema volume (ml).

## Results

### *Stimulation of Human Monocytes with LPS Increases Polyamine Uptake*

Preliminary experiments established that uptake of spermine, spermidine, and putrescine in monocyte cultures was linear over a 60-min period at 37°C; this accumulation was not observed at 4°C (not shown). The component of uptake at 4°C, reflecting binding or passive diffusion, was subtracted from the total uptake at 37°C, and this net amount was defined as specific polyamine uptake. In subsequent experiments, the accumulation of radiolabeled polyamines in monocyte

cultures was measured over a 25-min period. LPS induced a significant increase in spermine uptake compared to unstimulated, quiescent monocyte culture controls. As shown in Figure 1a, peak spermine uptake was observed 3 hr after exposure of monocyte cultures to LPS; by 18 hr, spermine accumulation declined to basal (unstimulated) rates. We next measured the effects of LPS on monocyte uptake of other polyamines (spermidine and putrescine) and observed a similar time course for LPS-induced uptake of these polyamines (not shown). Addition of spermine (10 μM) completely inhibited the uptake of spermidine (35 μM) and putrescine (35 μM), indicating that human monocytes express the nonselective polyamine transporter, which is sensitive to LPS stimulation.

Spermine uptake exhibited saturation kinetics as the concentration of spermine in the media

was increased (Fig. 1b). Wilkinson analysis of these concentration-rate data using PC Enzpack software indicated that the  $V_{\max}$  and  $K_m$  values describing the uptake of spermine in unstimulated human monocyte cultures were  $54.6 \pm 1.71$  pmol/25 min/ $10^6$  cells and  $1.15 \pm 0.185$   $\mu$ M, respectively. LPS stimulation significantly increased the  $V_{\max}$  value of spermine uptake to  $213 \pm 9.57$  pmol/25 min/ $10^6$  cells, but the  $K_m$  value ( $1.00 \pm 0.235$   $\mu$ M) was not significantly changed by LPS. Eadie-Hofstee plot analysis of spermine uptake was linear in both unstimulated and LPS-stimulated monocytes, suggesting that spermine interacted with a single transporter system.

#### *Inhibition of Monocyte Polyamine Uptake by BAP*

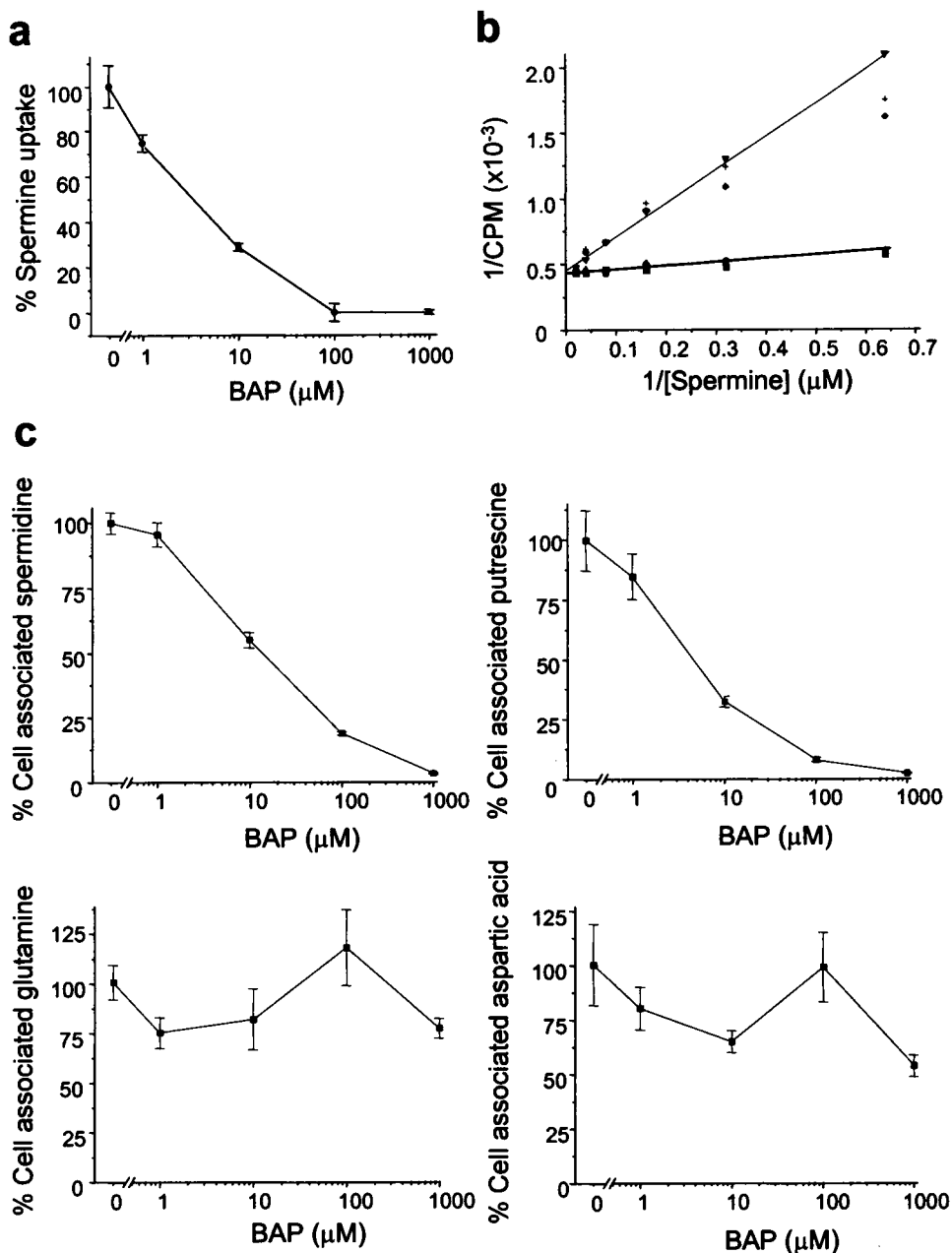
On the basis of our earlier observations that spermine deactivates macrophages (17), we considered it plausible that increased spermine uptake following monocyte activation might serve as a mechanism to prevent excessive macrophage activity. Our approach to study this hypothesis was to identify an inhibitor of spermine uptake in LPS-stimulated monocytes; the effects of this inhibitor on monocyte activation in vitro and monocyte-mediated tissue responses in vivo could then be examined. Accordingly, we screened a panel of spermine analogs for inhibition of spermine uptake in LPS-stimulated human monocyte cultures. One piperazine-like spermine analog, 1,4-bis(3-aminopropyl)-piperazine (BAP), decreased monocyte spermine accumulation in a dose-dependent manner; the 50% inhibitory concentration ( $IC_{50}$ ) of BAP was  $7 \pm 2.8$   $\mu$ M in the presence of 35  $\mu$ M spermine (Fig. 2a). BAP inhibition of spermine uptake was overcome by sufficiently high concentrations of spermine (Fig. 2b), giving direct evidence that BAP is a competitive inhibitor of monocyte spermine uptake. The inhibitory effect of BAP on spermine uptake was not due to cytotoxicity, because up to 1 mM of BAP did not suppress thiazoyl blue (MTT) metabolism in human monocytes (not shown). BAP also inhibited the uptake of spermidine and putrescine, but it did not inhibit the uptake of glutamine or aspartic acid in monocytes (Fig. 2c). Thus, BAP is a specific, competitive inhibitor of the LPS-inducible polyamine accumulation in human monocytes.

#### *Inhibition of Spermine Uptake Restores Monocyte TNF Synthesis*

We next measured TNF release from human monocytes in cultures supplemented with LPS and BAP. Addition of BAP alone did not activate monocytes to release TNF (Fig. 3); we also observed no significant effect of BAP on the magnitude of LPS-induced TNF release (not shown). BAP treatment, however, fully restored TNF synthesis in spermine-suppressed monocytes and the 50% effective concentration that restored TNF release in spermine treated cultures ( $EC_{50} = 8 \pm 3$   $\mu$ M) was comparable to the  $IC_{50}$  of BAP as an inhibitor of spermine uptake (Fig. 3). The TNF-enhancing activity of BAP in the presence of spermine was significantly correlated with the inhibitory activity of BAP against monocyte spermine uptake (correlation coefficient = 0.99;  $p < 0.05$ ). Putrescine shares the nonselective polyamine transporter with spermine but does not exert an inhibitory activity on monocyte cytokine synthesis (17). Given the requirement of spermine uptake for its inhibitory activity on monocyte TNF production, we reasoned that putrescine might also enhance cytokine production in spermine-suppressed human monocytes by competitively inhibiting the uptake of spermine. We therefore measured TNF release of spermine-treated human monocytes in the presence or absence of putrescine and observed a significant increase of TNF release by putrescine treatment in spermine-suppressed monocytes (not shown). Thus, with respect to inhibition of macrophage activation, spermine uptake is required for spermine inhibitory activity and increased spermine uptake in activated monocytes is a mechanism that acts to prevent monocyte over-activation.

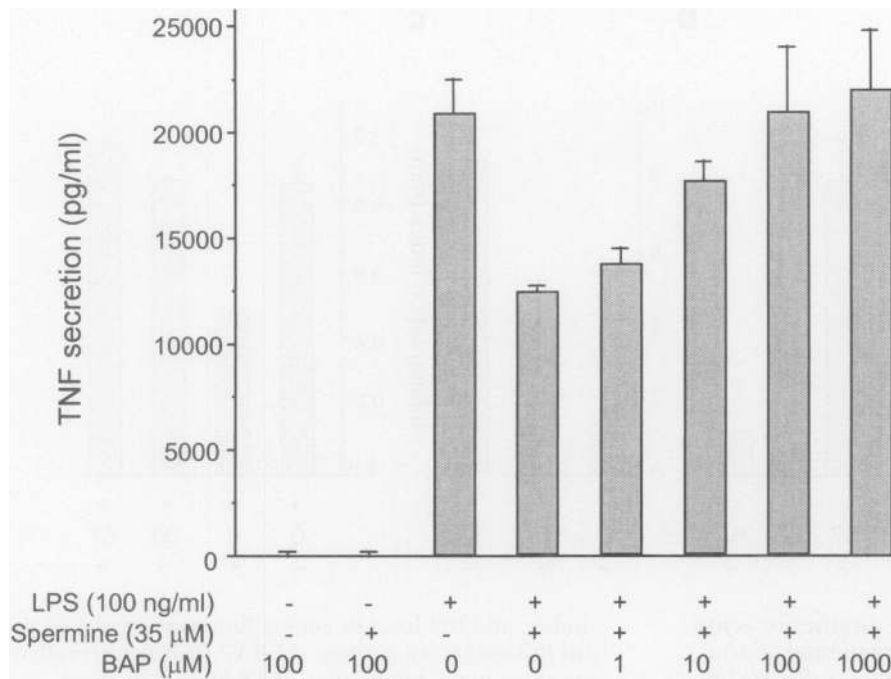
#### *Inhibition of Spermine Uptake Enhances Carrageenan-Induced Inflammation In Vivo*

It has been well documented that spermine levels are increased in various inflammatory states including infection (16,22,23), tissue injury (14,24,25), and cancer (15,26), suggesting a role of endogenous spermine in regulating the inflammatory process. We recently demonstrated that direct application of exogenous spermine into animal footpad suppresses carrageenan-induced paw edema (17), an inflammatory response that is predominantly mediated by macrophage products (e.g., TNF and NO) (27–29). While these results suggest an anti-inflammatory activity of spermine, it was previously unknown



**Fig. 2. BAP is a specific, competitive inhibitor of the nonselective polyamine transporter in human monocyte cultures.** (a) BAP inhibits spermine uptake in LPS-stimulated human monocytes. Human monocytes were stimulated by LPS (100 ng/ml) for 2 hr, then treated with BAP in the concentrations indicated for 30–60 min followed by 25 min incubation with 35  $\mu\text{M}$  spermine with  $^{14}\text{C}$ -labeled spermine tracer at either 4°C or 37°C. Spermine uptake (CPM) is defined by the difference between cell-associated radioactivity at 37°C and that at 4°C, and expressed as percentage of the control (no BAP) cultures. Results are percentage of control mean  $\pm$  SE. (b) Competitive inhibition of spermine uptake by BAP. Human monocytes were stimulated by LPS (100 ng/ml) for 2 hr, then incu-

bated with the indicated concentrations of spermine with  $^{14}\text{C}$ -labeled spermine tracer at either 4°C or 37°C for 25 min in the absence or presence of 500  $\mu\text{M}$  of BAP. Cell-associated radioactivity (CPM) was then measured, and spermine uptake (CPM) in human monocytes is shown as a reciprocal plot. The heavy line denotes spermine uptake in the absence of BAP, and the thin line, in the presence of BAP. (c) BAP inhibition of polyamine uptake is specific. Human monocytes were stimulated by LPS (100 ng/ml) for 2 hr, then incubated individually with  $^{14}\text{C}$ -labeled spermine, putrescine, glutamine, or aspartic acid at 37°C for 25 min in the absence or presence of BAP at the concentrations indicated. Cell-associated radioactivity (CPM) was measured and presented as percentage of control (no BAP)  $\pm$  SE.



**Fig. 3. Blockade of spermine uptake with BAP restores spermine-suppressed TNF synthesis.** Human monocytes were pretreated with spermine in the absence or presence of BAP at the indicated concentrations for 30–60 min, then stimulated by LPS (100 ng/ml) for 4 hr. Secreted TNF in cell culture supernatants was measured by ELISA. Results presented are the mean of TNF levels  $\pm$  SE.

whether endogenous spermine normally participates in the control of inflammatory responses. Accordingly, we evaluated the effects of BAP in carrageenan-induced paw edema by subcutaneous injection of BAP and carrageenan. Administration of BAP alone into rat foot pads failed to stimulate the development of edema in the absence of carrageenan, but coadministration of BAP with carrageenan significantly augmented carrageenan-induced foot pad inflammation (Fig. 4a). We also measured the levels of NO (total nitrite/nitrate) in the inflammatory paws and observed a significant increase of NO production in BAP-treated paws as compared with the controls (Fig. 4b). The direct involvement of spermine in BAP-enhanced excessive inflammation was supported by the observation that BAP treatment fully “restores” exogenous spermine-suppressed inflammatory responses, whereas additional exogenous spermine significantly reduced inflammation (Fig. 4c) typical of BAP treatment (Fig. 4a). Taken together, these results indicate that endogenous spermine can be incorporated into macrophages to restrain the innate inflammatory response.

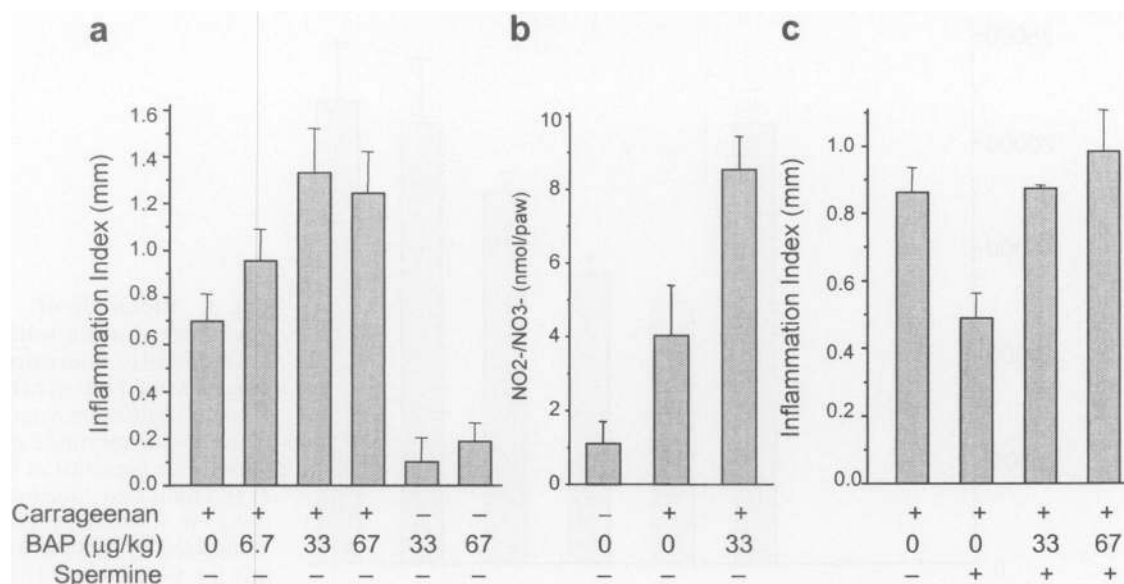
## Discussion

Four distinct lines of evidence from the present study indicate that human monocytes might use

a single nonselective transporter for the uptake of spermine. First, Eadie-Hofstee plot analysis of spermine uptake was linear, suggesting the presence of a single spermine transporter in human monocytes. Second, spermine treatment inhibits the uptake of putrescine and spermidine, indicating that the transporter for spermine is shared by putrescine and spermidine in human monocytes. This is further supported by the observation that putrescine effectively blocks the uptake-dependent effect of spermine on monocyte cytokine synthesis. Third, a similar time course for LPS-stimulated monocyte uptake of spermine, spermidine, and putrescine was observed, suggesting that LPS-stimulated uptake of polyamines is due to the enhancement of a nonselective transporter activity.

The fourth line of evidence is the inhibition of polyamine uptake by the piperazine-like spermine homologue BAP. BAP inhibited the uptake of all three polyamines by a similar magnitude but had no effect on the uptake of structurally unrelated molecules glutamine and aspartic acid. Thus, BAP is a specific inhibitor of the nonselective transporter in human monocytes. It has been well documented that polyamine transport is an integral part of the system that regulates intracellular polyamine levels according to various needs (20).

A role for monocyte polyamine transport activity, however, has not been previously re-



**Fig. 4. Administration of BAP augments acute inflammatory responses to carrageenan.** Carrageenan (0.2% in PBS) was injected into the left hind footpad of male Lewis rats in combination with the indicated concentration of spermine and/or BAP. Vehicle PBS was injected into the right hind footpad as control. Footpad thickness was measured 16–24 hr later, and the edema fluid was recovered. The difference (in mm) between the thickness of left and right hind footpad was defined as the inflammation

index, and NO level in edema fluid was measured as an inflammatory marker. (a) BAP treatment resulted in excessive inflammation. (b) BAP treatment increased NO production in carrageenan-induced paw edema. (c) The antagonistic interaction between BAP and spermine. Spermine reduces BAP-induced excessive inflammation, whereas the anti-inflammatory activity of exogenous spermine was blocked in the context of inhibition of spermine uptake by BAP treatment.

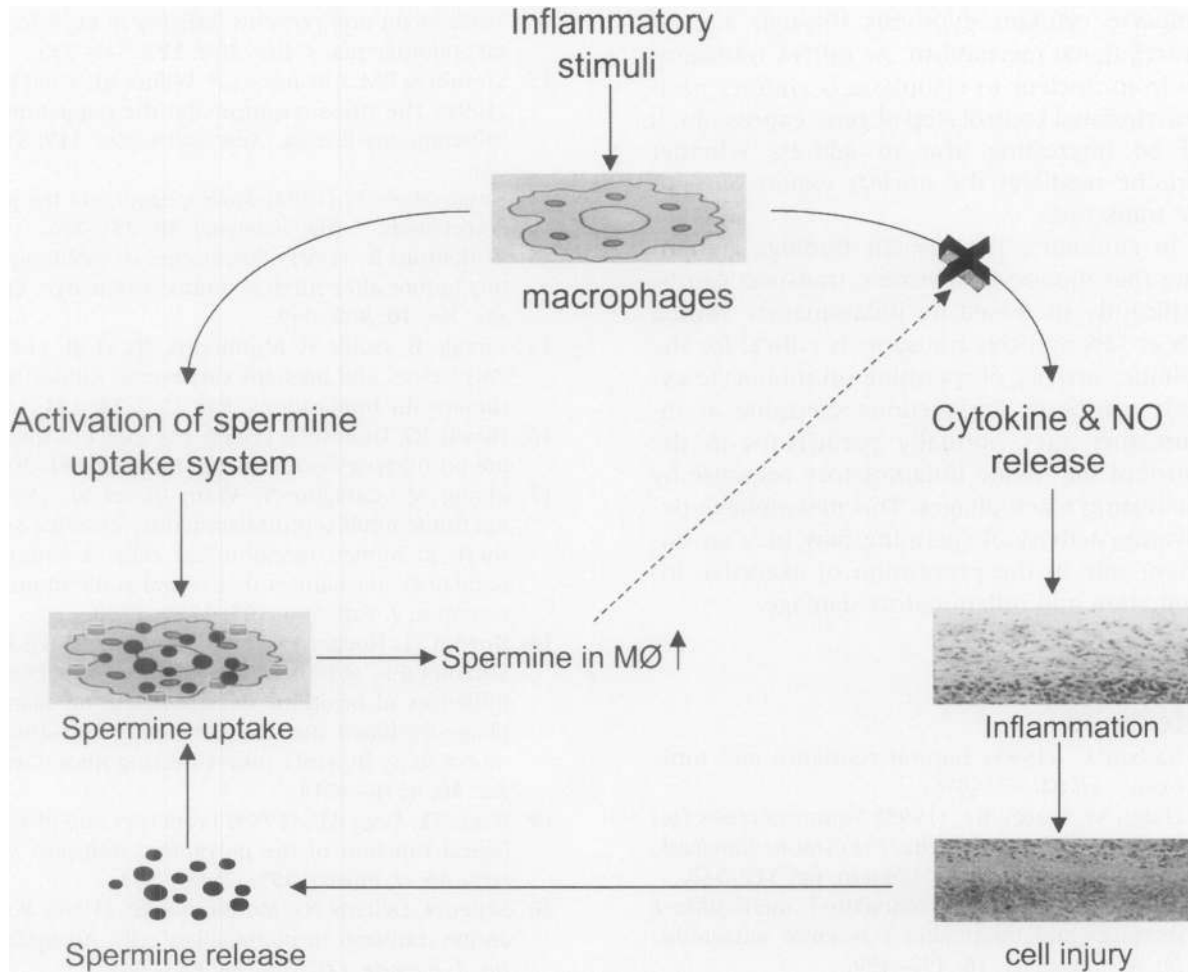
ported. An interesting observation in the present study is that monocyte spermine uptake is significantly increased following LPS stimulation. Inhibition of LPS-stimulated spermine uptake by BAP blocks the inhibitory activity of spermine on monocyte TNF production, and it displays a correlation coefficient of 0.99 ( $p < 0.05$ ) between the inhibitory activity of BAP on spermine uptake and the enhancing activity of BAP on monocyte TNF production. Thus, spermine uptake is required for spermine suppression of monocyte cytokine synthesis, and increased spermine uptake in activated monocytes provides a mechanism to prevent excessive monocyte activation. In agreement with the critical role of spermine in the regulation of monocyte activation, increased spermine uptake in activated monocytes is reversible and time dependent. Thus, monocytes can recover their activity in subsequent inflammatory responses when spermine is reduced or inactivated.

The activation of macrophages is a key event for the initiation and propagation of the innate inflammatory response. In the past, a number of molecules including IL-10, transforming growth

factor beta ( $\text{TGF-}\beta$ ), and glucocorticoids have each been implicated in preventing excessive macrophage activation and inflammation (8–13). Unlike IL-10,  $\text{TGF-}\beta$ , or the glucocorticoids, however, spermine is unique in being released locally from dying and injured cells (24). The quantity of spermine released at inflammatory sites will not only reflect the severity of ongoing tissue damage but also proportionally attenuate the innate inflammatory response (Fig. 5). This provides a critical restraining influence on inflammation, as the monocyte-deactivating effects of spermine prevent further release of TNF and NO, thereby limiting the extent of inflammation and associated collateral damage. In agreement with this proposed mechanism, mucosal polyamine deficiency has been implicated in the pathogenesis of inflammatory bowel disease (30).

Some 40 years ago, Hirsch and Dubos discovered increased local spermine levels at the site of mycobacteria infection (16). Subsequent studies in humans and animals indicate that spermine level is also increased in trauma (14,24,25), cancer (15,26), and other infections (22,23). It is





**Fig. 5. Regulation of macrophage activation and inflammation by spermine.** In response to infection, injury, and other inflammatory stimuli, host macrophages produce various factors, such as TNF, IL-1, and NO, that are critical for an effective immune and inflammatory response but can also be harmful or even lethal to the host at high levels. In response to inflammatory stimuli, the activity of the

spermine uptake system in activated macrophages is increased, leading to the incorporation of spermine from the surrounding environment. Spermine accumulation at inflammatory sites enhances intracellular macrophage uptake of spermine, resulting in the down-regulation of macrophage activation and inflammation.

interesting that the innate immune response is generally defective in these inflammatory conditions (31–33). Considering the anti-inflammatory activity of endogenous spermine, it is reasonable to consider that spermine accumulation in macrophages and monocytes may contribute to this immune dysfunction. It will be interesting to test whether the blockade of spermine activity with spermine antagonists like BAP can restore the innate immune response in these conditions.

The data presented in this work indicate that cellular uptake of spermine is required for the inhibitory effect of spermine on monocyte cytokine synthesis. Recent evidence (34) indicates that fetuin, a negative acute-phase protein, is

required for spermine inhibition on monocyte cytokine synthesis, because anti-fetuin antibodies prevent the cytokine-inhibiting effects of spermine. The relation between fetuin and the monocyte uptake of spermine is unknown. Immunoelectron microscopic studies (35) have revealed that intracellular spermine is predominantly located on ribosomes of the rough endoplasmic reticulum, and it is one of the components of biologically active ribosomes. It is therefore possible that spermine might suppress monocyte cytokine synthesis by regulating monocyte cytokine mRNA translation or mRNA stabilization. In agreement with this, our previous studies (17) show that spermine suppresses

monocyte cytokine synthesis through a post-transcriptional mechanism. As mRNA translocation from nucleus to cytoplasm is another post-transcriptional control step of gene expression, it will be interesting also to address whether spermine regulates the nuclear export of cytokine transcripts.

In summary, the present findings demonstrate that monocyte spermine transport can be significantly increased by inflammatory stimuli such as LPS and this transport is critical for the inhibitory activity of spermine on monocyte cytokine synthesis. Endogenous spermine at inflammatory sites normally participates in the control of the innate inflammatory response by deactivating macrophages. This macrophage-deactivating activity of spermine may play an important role in the prevention of excessive inflammation and inflammatory damage.

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