On the expression of nitric oxide synthase by human macrophages. Why no NO?

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Abstract: The production of nitric oxide (NO) and its role in the anti-tumor and anti-microbial effects of rodent macrophages appears well established. In contrast, the circumstances required for its release from human monocytes/macrophages and its potential role in human pathology remain controversial. Evidence to be discussed suggests that NO is a redundant, autotoxic, immunosuppressive, and inefficient mediator of macrophage function. For these reasons, the expression of nitric oxide synthase as a rapid-response, high-output effector pathway may have been evolved out of the human monocyte/macrophage response repertoire or severely restricted in its expression. Hypothetical roles for a modest and circumscribed production of NO by human macrophages are proposed. *J. Leukoc. Biol.* 58: 643–649; 1995.

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Interest in the participation of the amino acid L-arginine in immune responses has experienced a rebirth since the discovery of the nitric oxide synthases, more specifically that of the inducible form of this enzyme (iNOS) in rodent macrophages. In this connection, a role for nitric oxide (NO) as a mediator of anti-tumor and anti-microbial effects of macrophages has been proposed, discussed, and reviewed in an ever growing number of publications [1].

In a previous incarnation as a substrate for arginase, L-arginine experienced its Warholian 15 minutes of fame in the late 1970's as a mediator of some of the same anti-tumor and anti-microbial activities of rodent macrophages now credited to NO. It was proposed that arginase, secreted from activated macrophages, consumed L-arginine both in vitro and in vivo (reviewed in ref. 2). The extensive catabolism of L-arginine through arginase, in turn, reduced the local availability of this amino acid to the extent that it became rate limiting for the proliferation and survival of the targeted tumor cells or microorganisms and, in striking resemblance to a known effect of NO [3], for lymphocyte proliferation.

Although more than 40 publications addressed arginase as an effector molecule of macrophages, few actually investigated the presence of this enzyme in human monocytes or macrophages. Cameron et al. [4] and Scheemann et al. [5], in this regard recently showed that neither monocytes nor alveolar or peritoneal macrophages obtained from humans express arginase activity in culture. It appears, then, that the involvement of arginase in macrophage-dependent immune responses is species restricted.

In a similar manner, most work on NO as a mediator of macrophage toxicity against tumors and microorganisms has been performed using cells obtained from rats and mice. Although rodent macrophages and macrophage cell lines derived from them [6] are easily induced to express NOS activity, demonstrating high-output NO production by human monocytes/macrophages, either directly or through the appearance of its catabolites citrulline, nitrites or nitrates, has encountered significant difficulties.

That human monocytes/macrophages contain and can express the iNOS gene is indubitable. The accumulation of iNOS mRNA and/or protein has been documented in interferon-γ (IFN-γ) and lipopolysaccharide (LPS)-stimulated human monocytes and in those cultured with HIV-1 [7–9]. The distillate of published data indicates, however, that the actual production of NO by human monocyte/macrophages differs markedly from that by macrophages harvested from rats or mice. Most specifically, while treatment of rodent macrophages with classical soluble activating molecules like IFN-γ, tumor necrosis factor α (TNF-α), or LPS results in the rapid and abundant production of NO (**Table 1**), these signals have mostly failed to induce the appearance and accumulation of NO degradation products in cultured human monocytes/macrophages [4, 5, 7, 9–19]. It appears that the induction of iNOS in human monocytes/macrophages, under the best of circumstances, requires a period of stimulation of several days' duration [8, 20–22], that stimulation by some [20, 23] but not all [4] microorganisms or specific tumor cells [18] is seemingly required for enzyme activity in vitro, and, most importantly, that the actual amount of NO metabolites released by human monocytes/macrophages is, by most reports, remarkably modest when compared with their production by rodent cells (Table 1). More than the high-output, rapid-response effector mechanism described in mice and rats, the human macro-

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Abbreviations: NO, nitric oxide; iNOS, inducible form of NO synthase; IFN-γ, interferon-γ; LPS, lipopolysaccharide; TNF-α, tumor necrosis factor-α; *N*-MMA, N^G-monomethyl-L-arginine; IL-4, interleukin-4.

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TABLE 1. Positive Reports on the Production of NO by Human Monocytes/Macrophages

Author (Ref)	Stimulus	NO_2^- production
Denis [20]	*M. avium*[a]	102 ± 18 μM/10^6 cells/7 days[a]
	M. avium + TNF-α[b]	211 ± 20 μM/10^6 cells/7 days[a,c]
Muñoz-Fernandez [23]	*T. cruzi* (?)[d]	4 μmol/10^6 cells/72 h
	T. cruzi + TNF-α + IFN-γ[d]	55 μmol/10^6 cells/72 h[e]
Mautino [32]	None	≡3 nmol/10^6 cells/24 h[f]
Kolb [31]	IL-4[g]	≡0.6 nmol/10^6 cells/24 h[h]
Zembala [18]	Tumor cells[i]	≡25 nmol/10^6 cells/48 h[j]
De Maria [11]	CD69 X-linking	≡3 nmol/10^6 cells/24 h[k]
Bukrinsky [8]	HIV-1[l]	≡1 nmol/10^6 cells/24 h[m]
Dumarey [24]	None	18 ± 3 nmol/10^6 cells/24 h[n]
	M. avium	41 ± 3 nmol/10^6 cells/24 h[n]
Martin [22]	None	12.5 nmol/10^6 cells/24 h[o]
Martin [12]	None or IFN-γ	26 nmol/10^6 cells/24 h[p]
Rat peritoneal macrophages	None	5.9 ± 0.5 nmol/10^6 cells/24 h
	IFN-γ/LPS[q]	25.4 ± 0.6 nmol/10^6 cells/24 h
Mouse peritoneal macrophages	None	0.4 ± 0.2 nmol/10^6 cells/24 h
	IFN-γ/LPS[q]	18.3 ± 1.7 nmol/10^6 cells/24 h

Most reports use the accumulation of NO_2^- in culture supernatants as an indicator of production of NO. Nitrate generation was, as far as it can be found in the reports summarized here, only measured by Dumarey [24]. None of the reviewed studies used radiometric methods to simultaneously determine citrulline production. Unfortunately, the means of reporting NO_2^- production by cultured cells varies greatly among publications. Few actually indicate the rate of NO_2^- production or accumulation, with most reports only containing the final concentration of NO_2^- in culture supernatants. Since culture volumes, duration of culture, or cell numbers are not universally indicated, comparison of the rate of NO_2^- (or NO_3^-) release among different publications becomes quite difficult. Data reported in this table were calculated from information available in the indicated references. Production of NO_2^- by rat or mouse resident peritoneal macrophages is provided for comparison.

[a] NO_2^- production reported as μM(?)/10^6 cells/7 days. Unable to calculate rate of NO_2^- formation from data in manuscript. Viable cells at 7 days were 60% of original number plated. It appears that cells were cultured for 7 days in the presence of *M. avium*. This is concluded from the legend to one of the tables in the paper but not specifically indicated in Methods.

[b] TNF-α added at 100 units/ml.

[c] *N*-MMA (1 mM) inhibited NO_2^- production by >90%.

[d] Unclear from the text whether all cells were exposed to the parasite or whether *T. cruzi* were present only in cultures containing cytokines. TNF-α (300 U/ml) and IFN-γ (100 U/ml) were presented to the cells for 4 h before adding parasite and removed 12 h later.

[e] *N*-MMA (100 μM) suppressed NO_2^- production by 60%.

[f] Data for high responders calculated from author's figures between days 6 and 8 of culture. No NO_2^- was detectable in the cultures up to day 4 in culture.

[g] IL-4 added at 200 U/ml.

[h] Calculated from author's figure between days 6 and 12 in culture. Data for "responder" subjects. Addition of IFN-γ to cultures at 500 U/ml increased NO_2^- release by >100%.

[i] Effect observed only with DeTa cells and not with other tumor cells.

[j] Calculated from authors' figures. Monocytes and tumor cells co-cultured for 48 h. NO_2^- production was suppressible by *N*-MMA or by preculturing the monocytes with Leu-O-Me, emetine, or actinomycin D.

[k] NO_2^- detected at 24 h culture.

[l] Peripheral blood monocytes were cultured for 7 days with granulocyte-macrophage colony-stimulating factor (1000 U/ml) and then exposed to HIV-1.

[m] Calculated from authors' figures between days 6 and 7 in culture post-HIV-1 exposure. Nitrite production was enhanced ~threefold by TNF-α (1000 U/ml) or LPS (up to 100 ng/ml) and partially suppressed by *N*-MMA at 2 mM or IL-4 at 5 ng/ml.

[n] Total NO_2^- + NO_3^- produced, measured as NO_2^-. *N*-MMA suppressed *M. avium*-induced NO_2^- + NO_3^- release by ~50%.

[o] Apparent rate of release for initial 24 h in culture. Data indicate a marked increase in amount of NO_2^- in culture supernatants between culture days 5 and 6. Unable to calculate rate of release since it cannot be discerned from data whether NO_2^- amounts reported are cumulative or per day.

[p] Apparent rate of release for initial 24 h. Amount of NO_2^- in culture supernatants is shown to increase progressively over a 6-day culture. Unable to calculate rate of release since it cannot be discerned from the data whether NO_2^- amounts reported are cumulative or per day.

[q] Rat or mouse resident peritoneal macrophages were cultured at 10^6/ml in media containing 1 mM L-arginine and, when so indicated, IFN-γ (10 U/ml) and LPS (from *E. coli* 055:B5, 1 μg/ml). Accumulation of NO_2^- in supernatants was measured after 24 h culture.

phage iNOS appears to be a relatively low-output, slow-burn system.

Four apparent exceptions to the slow-induction and/or low-output components of this paradigm can be found in the literature. Denis [20] first reported that human monocytes produced large amounts of NO_2^- in culture when cultured with *M. avium* for 7 days. Dumarey et al. [24] showed a moderately high rate of production of NO_2^- + NO_3^- by unstimulated monocytes (18 ± 3 nmol/10^6 cells/day) and indicated that viable, but not killed, *M. avium* induced the increased production of reactive nitrogen intermediates (to 41 ± 3 nmol/10^6 cells/day). Contrasting with these observations, Bermudez [15] failed to induce the production of NO in murine or human macrophages using live *M. avium* or *L. monocytogenes*. Martin and Edwards [12, 22] indicated that unstimulated cultured human peripheral blood monocytes produce relatively high levels of NO_2^- (12.5 nmol/10^6 cells/day [22] to 26 nmol/10^6 cells/day [12]) during the first day of culture, and that production increases linearly [12] with time or abruptly [22] after 6 days in culture. Lastly, De Maria et al. [11] recently published a report that cross-linking of the CD69 cell surface receptor in human monocytes resulted in the modest but rapid production of NO_2^- by the cells (~3 nmol/10^6 cells/day for the first 2 days in culture). The authors also showed that the production of NO_2^- correlated with the monocytes' ability to kill L929 and P815

tumor cells in vitro. These data are somewhat difficult to interpret. First, the characterization of L929 cells as NO-sensitive contrasts directly with reports of the NO-resistant nature of these cells [25–27]. In addition, data of De Maria et al. [11] appear to indicate an extraordinary sensitivity of P815 cells to human monocyte-derived NO. In this connection, the authors report ~40% P815 tumor cell killing by the end of an 18-h tumor cell/macrophage co-culture at an effector-to-target ratio of 24:1. The amount of NO produced by the macrophages included in these cultures can be calculated from the authors' figures to have been ~0.6 nmol. In contrast, murine peritoneal macrophages activated with IFN-γ and LPS and co-cultured with P815 cells at an effector-to-target ratio of 20:1 produced ~50 nmol NO_2^- and resulted in only 15% specific tumor killing over the same period of time [25]. These results could be interpreted to indicate that NO produced by human macrophages is somehow more efficient in killing P815 than that made by rodent macrophages or, vide infra, that a co-cytotoxic molecule is produced by the effectors simultaneously with NO.

Some reasonable explanations for the difficulties in demonstrating high-output NO production by human macrophages have been put forth and can probably be discarded. Human monocytes/macrophages cannot synthesize tetrahydrobiopterin, an essential co-factor for iNOS activity. This metabolic handicap should present an insurmountable restriction to these cells' ability to produce NO. It has been proposed in this regard that monocytes or macrophages could acquire the necessary reduced biopterin from other cells, such as lymphocytes [4]. No evidence to support this hypothesis has been reported. At any rate, the inability of human monocytes to generate tetrahydrobiopterin does not appear to be the rate limiting factor for the production of NO by these cells, since loading with tetrahydrobiopterin precursors failed to support stimulated NO_2^- release from human monocytes [16, 19].

Differences in maturation or differentiation between the peritoneal rodent macrophages commonly used for the study of iNOS and human peripheral blood monocytes do not seem to explain the difficulties mentioned previously in inducing iNOS in the latter. This follows from studies where human alveolar or peritoneal macrophages failed to produce NO when stimulated with cytokines and endotoxin or cytokines and microorganisms [4, 5, 28].

That human macrophages produce NO metabolites different from the commonly measured NO_2^- or NO_3^- was negated by studies demonstrating that these cells are equally incapable of producing L-citrulline, an additional product of the metabolism of L-arginine through NOS (refs. 4 and 5; J. Albina, unpublished observations). In this connection, it appears relevant to highlight the recent report by Zinetti et al. [29] that culture with N^G-monomethyl-L-arginine (*N*-MMA), a competitive inhibitor of NOS, or hemoglobin reduces the release of TNF-α from LPS-stimulated human monocytes in a 4-h culture. Interestingly, the LPS-treated cells did not release measurable NO_2^- or NO_3^- into culture supernatants. Since hemoglobin can obviously only act extracellularly, this report suggests that a NO metabolite different from NO_2^- or NO_3^- is most rapidly released from the cells and, in an autocrine fashion, feedback-inhibits TNF-α production by the cells.

Importantly, if signal-restricted expression and low NO output are canonical features of the human macrophage iNOS, these are not a necessary feature of all human iNOS. The rapid induction of iNOS in human hepatocytes by cytokines and the abundant production of NO by these cells [30] argues against the existence of regulatory factors that, present in all human cells, may explicate the peculiarities of NO release from human macrophages.

Even when NO production has been successfully induced in human monocytes/macrophages, there appears to be significant interindividual variability in the capacity of these cells to make NO in culture. In this connection, Kolb et al. [31] found that monocytes from three out of seven donors produced small amounts of NO when stimulated with interleukin-4 (IL-4). Preincubation with IFN-γ resulted in positive responses to IL-4 in cells from all subjects. Intriguingly, work from the same laboratory indicated that IL-4 enhanced NO production in cells from normal donors but inhibited its release by cells obtained from allergic donors [32]. Compounding the complexity of this issue, Bukrinsky et al. [8] recently reported that IL-4 suppressed NO production by human macrophages cultured with HIV-1, TNF-α, and LPS while, parenthetically, indicating that the production of NO in response to HIV-1 occurred in cells from four out of six subjects. In addition then to the subject-dependency of NO production by human monocytes/macrophages, IL-4, which suppresses NOS in murine macrophages, can apparently promote [31, 32] or inhibit [8, 32] NO release from human macrophages.

A recent publication by Weinberg et al. [19] on the production of NO by human monocytes and peritoneal macrophages deserves a separate paragraph. Findings reported in this sweeping survey can be summarized as follows: (1) peripheral blood monocytes harvested from a rather large number of donors (23–74 depending on culture conditions) mostly failed to produce NO_2^-/NO_3^- during 3- to 4-day culture with a variety of putative inducers of iNOS including cytokines and growth factors (IFN-γ, TNF-α, IL 1, 2, 4, 6, or 7 and granulocyte-macrophage colony-stimulating factor), endotoxin, 1,25 dihydroxyvitamin D_3, phorbol myristate acetate, A23187, concanavalin A, phytohemaglutinin, *L. monocytogenes*, *C. albicans*, *S. epidermidis*, *M. avium complex*, *M. tuberculosis*, or HIV-1-Bal. Just as reported by others [8, 31] cells from an "occasional" donor were capable of NO_2^-/NO_3^- release. (The frequency of this occurrence is not reported in the study.) (2) Peritoneal macrophages obtained during laparoscopic examination of women undergoing diagnostic work-up for infertility released some NO_2^-/NO_3^- in culture. The production of NO_2^-/NO_3^- by these cells apparently was enhanced by endotoxin and/or IFN-γ. (Unfortunately the authors do not indicate the number of cells cultured nor the cell culture volumes. As mentioned elsewhere in this review, this makes it impossible to calculate the rate of NO production by the cells.) (3) As indicated by others [16], neither monocytes nor macrophages

contained tetrahydrobiopterin. Lysates from monocytes and macrophages converted L-arginine to L-citrulline in an *N*-MMA-inhibitable manner, with lysates from LPS ± IFN-γ-treated macrophages producing more L-citrulline than unstimulated cells. The capacity of human cell lysates to produce L-citrulline was, however, at best approximately two logs less than that of the J774 murine macrophage cell line. (4) Inducible NOS mRNA was detected by RT-PCR in both human cell types under study after 8 h culture with LPS and/or IFN-γ. These cells also contained protein immunoreactive to an antibody against the murine iNOS. (5) Last, the discrepancy between the relative abundance of immunoreactive iNOS protein in human cells and their limited capacity to generate NO_2^-/NO_3^- triggered experiments designed to identify a functional inhibitor of the enzyme. To detect the presence of such an inhibitor, extracts from LPS ± IFN-γ-treated human monocytes were mixed with those from activated J774 cells. No inhibition of L-arginine conversion to L-citrulline was observed in these experiments.

As indicated so far, the pattern that emerges from the literature is that human monocytes/macrophages appear to be particularly reluctant to produce NO. They are not alone. Neither rabbit monocytes [5], peritoneal [5] or alveolar [4] macrophages, nor peripheral blood monocytes from new- or old-world monkeys (Albina, unpublished observations) produce NO in culture when stimulated with IFN-γ and/or LPS. In contrast, bovine bone marrow- and monocyte-derived macrophages have been recently shown to accumulate iNOS mRNA and secrete nitrites when stimulated with *S. dublin* or with endotoxin, but not when treated with IFN-γ, IL-2, or IL-1 [33].

Important species differences in the expression and regulation of iNOS are evident even when comparing macrophages harvested from mice to those obtained from rats (Table 1). Unstimulated murine peritoneal macrophages metabolize L-arginine almost exclusively through arginase and express iNOS only after appropriate stimulation [34, 35]. In contrast, unstimulated rat peritoneal macrophages process L-arginine almost exclusively through NOS in culture even though they also contain arginase [34].

Establishing the mechanisms for differences among species in the production of NO by monocytes and macrophages provides a fascinating subject for investigation. If indeed unidentified essential cofactors or inhibitors explain the tight regulation of NOS in human monocytes/macrophages [1], their identification will shed light on the precise physiological or pathological circumstances where this enzyme could be expressed by human immune cells. Be that as it may, however, the fact remains that the expression of NOS by human monocytes/macrophages appears highly restricted. Working, then, from current knowledge it may be useful to speculate on hypothetical evolutionary pressures for a restricted expression of iNOS in human macrophages.

First, human monocytes/macrophages can apparently carry out their assigned tasks without resorting to the production of NO. Summarized in **Table 2** are results from studies investigating the NO-dependence or -independence of specific antimicrobial effects of human and rodent macrophages. These data indicate that, for the most part, human monocyte/macrophages exert effective antimicrobial activity without resorting to NO as a mediator. Exceptions to this conclusion are observations reported by Muñoz-Fernandez et al. [23] who found that human monocyte infection with *T. cruzi* and stimulation with IFN-γ gave rise to a significant amount of NO_2^- and to the NO-dependent killing of the parasite.

TABLE 2. Species Dependence of the Involvement of NO in the Antimicrobial Effects of Macrophages

	NO-dependent antimicrobial action	NO-independent antimicrobial action
Cryptococcus	Mouse [61]	Human [4]
Leishmania	Mouse [62]	Human [17]
Toxoplasma	Mouse [63]	Human [17]
Schistosoma	Mouse [64]	Human [14]
Listeria	Mouse [57]	Human [5, 15]
Trypanosoma	Human [23]	

Evidence for species differences in the NO-dependence or -independence of antimicrobial activity of monocytes/macrophages. Numbers in brackets are references.

Second, the production of NO may be counterproductive. It has been shown, in this connection, that NO generation renders murine and rat macrophages suppressive for alloantigen- and mitogen-driven T cell proliferation and suppresses antibody production [3, 36–41]. Moreover, Gregory et al. [42] recently showed that the inhibition of NO production through the administration of *N*-MMA to *L. monocytogenes*-infected mice reduced the number of viable *Listeria* in the liver 7 days postinfection by 100-fold. In addition, these authors reported that NO suppresses the antigen-specific blastogenic response to *Listeria* by splenic T cells isolated from infected animals. In an observation to be discussed later in more detail, Gregory et al. [42] indicated that, although the treatment of hepatocytes with cytokines and endotoxin promoted iNOS expression and *Listeria* killing, inhibiting NOS with *N*-MMA did not alter microbicidal activity. The production of NO obviously paralleled the development of cidal activity but was, in this context, irrelevant to the killing event.

Data from Liew et al. [43] offer a contrasting view of the potential importance of NO in controlling infection in vivo. These authors reported that the injection of *N*-MMA into *Leishmania major*-infected mouse footpads resulted in a dramatic increase in recoverable live parasites. The mechanism for these findings, however, need not involve macrophages as the NO generators mediating leishmanicidal capacity. Indeed, *N*-MMA could have altered the inflammatory response through alterations in local blood flow [44], local oxygen availability [45], or other nonimmune mechanisms. At any rate, it can be proposed that the indiscriminate immunosuppressive effects of NO may outweigh its value as a direct antimicrobial effector.

A third consideration involves the toxicity of NO and/or its metabolites [46, 47] for the NO-producing cells and the surrounding host tissues. Evidence has been presented that NO production is associated with decreased superoxide re-

lease, phagocytosis, and protein synthesis in rodent macrophages [34, 48] and that the continued production of NO by these cells is followed by their apoptotic death [49]. Suggesting that NO may be the ultimate double-edged sword, its production during immune/inflammatory responses goes hand-in-hand with tissue injury [50, 51]. This was shown, for example, in experimental models of arthritis where the administration of NOS inhibitors greatly curtailed joint damage [50].

In regard to potential detrimental effects of NO production during human disease, Brukinsky et al. [8] proposed that NO generated by HIV-1-infected human macrophages could be responsible for neurotoxicity in AIDS through injury to adjacent brain tissue. Whether the modest amounts of NO produced by the infected macrophages would be quantitatively sufficient to damage neighboring cells remains to be determined.

NO is not alone in being both a mediator of macrophage function and of tissue injury. Evidence for toxic effects of the reactive oxygen intermediates produced by rodent and human monocytes/macrophages has been convincingly presented [52–54]. Interestingly, the ability to produce large amounts of reactive oxygen intermediates by immune cells is generally paralleled by their capacity to curtail their autotoxic effects through specific molecules (i.e., superoxide dismutase, catalase, glutathione, etc.). In a similar fashion, immune cells producing large quantities of NO contain the only other high-capacity mammalian enzyme capable of metabolizing L-arginine, namely arginase. In this regard, arginase is present in macrophages obtained from rats and mice [55] but not in human monocytes/macrophages [4, 5]. Indeed, human monocytes/macrophages plainly fail to catabolize significant amounts of L-arginine in culture (refs. 4 and 5; Albina, unpublished observations). The importance of arginase as an inhibitor of NOS through competition for substrate is supported by findings in rat wounds where arginase derived from macrophages and contained in wound fluid maintains the extracellular free L-arginine concentrations at almost undetectable levels and appears to prevent the local expression of NOS [55, 56].

Last, NO may not even do what it is supposed to do. Although it has been shown, for example, that the production of NO by murine macrophages correlates with the killing of *Listeria* [57], mentioned earlier were findings by Gregory et al. [42] demonstrating that the induction of listericidal capacity in hepatocytes by cytokines and endotoxin was coincident with, but not dependent on, the production of NO by the cells. In the same manner, recent results indicate that macrophages harvested from experimental wounds in rats, while capable of producing NO in quantities comparable to those made by tumoricidal *C. parvum*-elicited peritoneal macrophages, are incapable of killing NO-sensitive P815 cells (Albina, unpublished observations). These observations are echoed by those of Tanaka et al. [58] who elegantly showed that macrophages harvested from FN-IL-6 (-/-) mice are capable of producing NO in the same quantities as cells from wild-type animals but are unable to kill *Listeria monocytogenes* or P815 cells. However, similar to results obtained using rat macrophages [34], they found that the bactericidal and tumoricidal activity of macrophages from wild-type animals was inhibitable by *N*-MMA. Taken together, these results suggest the possibility that the release of NO by macrophages coincides and is related in some way to the production of a co-cytotoxic molecule or molecules, but not solely responsible for the killing of microorganisms or tumor cells.

Based on available data, then, a tenable working hypothesis is to propose that NO is not liberally utilized by human monocytes/macrophages as a rapid-response, high-output effector pathway. Evolutionary pressure to avoid the production of large amounts of NO may have been provided by the redundant, immunosuppressive, autotoxic, and inefficient characteristics of NO as a macrophage effector molecule. It is, following this line of reasoning, tempting to speculate on the potential circumstances where a slow-response, low-output NO-producing system could indeed mediate human monocyte/macrophage function. Once such circumstance could occur during retroviral infections. It has been shown, in this regard, that peak production of NO by HIV-1-infected human monocytes/macrophages was followed by an immediate and profound decrease in reverse transcriptase activity [8]. At significant risk of overinterpreting these data, could it be possible that the general inhibitory effects of NO for protein synthesis be suppressive for viral replication? In this connection, the different relative sensitivities of the multiple molecular targets of NO to this mediator have been highlighted by Lancaster [59]. More specifically, while Drapier et al. [60] indicated that the inhibition of aconitase by NO occurs only after a threshold amount of NO is exceeded, total protein synthesis by macrophages is inhibited linearly with the production of NO [48]. Low-output NO production by virally infected human monocytes/macrophages may suffice to inhibit protein synthesis and, therefore, viral replication, while avoiding other effects of NO that may be toxic for the cells and, presumably, require higher concentrations of this effector.

Results to date indicate that the rapid and massive production of NO through NOS, like the expression of arginase, has been selected against as an indiscriminate effector mechanism for the tumor-cytotoxic and antimicrobial activity of human monocyte/macrophages. Whether some more subtle function can be found for NO in mediating macrophage function in humans remains to be seen. Research efforts should focus on detecting circumstances, if any, where the specific characteristics of the human monocyte/macrophages iNOS can be used beneficially by the host.

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