Nitrosative Capacity of Macrophages Is Dependent on Nitric-oxide Synthase Induction Signals*

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Nitrosative stress can occur when reactive nitric oxide (NO) species compromise the function of biomolecules via formation of NO adducts on critical amine and thiol residues. The capacity of inducible nitric-oxide synthase (iNOS) to generate nitrosative stress was investigated in the murine macrophage line ANA-1. Sequential activation with the cytokines IFN-γ and either tumor necrosis factor-α or interleukin-1β resulted in the induction of iNOS and production of nitrite (20 nm/min) but failed to elicit nitrosation of extracellular 2,3-diaminonaphthalene. Stimulation with IFN-γ and bacterial lipopolysaccharide increased the relative level of iNOS protein and nitrite production of ANA-1 cells 2-fold; however, a substantial level of NO in the media was also observed, and nitrosation of 2,3-diaminonaphthalene was increased greater than 30-fold. Selective scavenger compounds suggested that the salient nitrosating mechanism was the NO/O2 reaction leading to N2O3 formation. These data mimicked the pattern observed with a 5 μM concentration of the synthetic NO donor (Z)-1-(N-ammoniopropyl)-N-(n-propyl)amino[diazen-1-ium-1,2-diolate (PAPA/NO). The NO profiles derived from iNOS can be distinct and depend on the inductive signal cascades. The diverse consequences of NO production in macrophages may reside in the cellular mechanisms that control the ability of iNOS to form N2O3 and elicit nitrosative stress.

Nitric oxide (NO)1 is a unique molecule that regulates numerous cellular functions solely through its chemical reactivity with the surrounding milieu. The formation of NO is catalyzed by a diverse family of nitric-oxide synthase (NOS) isoenzymes (1–6). Activation of the immune system can result in the expression of the inducible isoenzyme iNOS in numerous cell types (e.g. macrophages, neutrophils, glia, and hepatocytes). Although iNOS has been implicated in a diverse array of pathologic events, the relative contributions of NO and derived reactive nitrogen oxide species to disease processes are often unclear. Moreover, whether immune-stimulated cells can regulate the reactivity of NO per se by shifting the balance between NO and its various intermediate reaction partners, thereby altering subsequent effector interactions of nitrogen oxide species with target biomolecules, remains unanswered.

In this study, we address these issues in the context of nitrosative stress, a condition often opposing oxidative stress (7–9), that may be a major factor in predicting the role iNOS plays during inflammation and disease.

In nitrosation reactions, N-nitrosamines and S-nitrosothiols are formed by the addition of a nitrosonium equivalent (NO+) to amine and thiol moieties, respectively (10). Activation of rodent macrophages with both IFN-γ and LPS results in iNOS expression and nitrosation of target molecules present in the culture medium (11–15). Several iNOS-dependent reaction pathways have potential relevance in the nitrosation chemistry of biological systems. Acidified nitrite (HNO2) is a nitrosating agent, but it is formed only in low pH environments, such as that of the stomach (10, 15). The mobilization of nonheme iron nitrosyl complexes from macrophages and hepatocytes activated with both cytokines and LPS (16, 17) may provide a mechanism for nitrosation, in particular, trans-S-nitrosation (18). Dinitrogen trioxide (N2O3) formed by the reaction of NO with molecular oxygen (O2) has a strong propensity to nitrosate both amine and thiol moieties at physiologic pH (19). The present study examined the nitrogen oxide profiles of a murine macrophage cell line to determine the conditions that may result in nitrosative stress on the extracellular milieu. The data show that macrophages produce a nitrosating agent consistent with the chemical profile of N2O3 (14); however, this phenotype was dictated by the inductive stimuli produced by the macrophage.

EXPERIMENTAL PROCEDURES

Culture Conditions—The murine macrophage cell line ANA-1 was cultured in Dulbecco’s modified Eagle’s medium (Life Technologies, Inc.) supplemented with 10% heat inactivated fetal bovine serum (HyClone, Logan, UT), 4.5 g/liter glucose, 2 mM l-glutamine, 100 units/ml penicillin, and 100 μg/ml streptomycin at 37 °C in a humidified incubator with 5% CO2 in air. Cells were trypsinized and seeded in 24-well plates at a density of 4 × 10^5 in a 1-ml volume of Dulbecco’s modified Eagle’s medium (85% confluence). A two-step stimulation paradigm was used: 1) overnight incubation with INF-γ (100 units/ml; R&D Systems, Minneapolis, MN), followed by 2) the addition of 20 ng/ml of either TNF-α, IL-1β (R&D Systems), or LPS (E. coli, 0111:B4; Sigma) and a second incubation period of 4 h. Subsequently, the wells were aspirated and gently rinsed with phosphate-buffered saline (pH 7.4, 37 °C). Cells were incubated in an assay buffer consisting of a phosphate-buffered saline solution supplemented with l-arginine (1 mM), diethylenetriamine pentaacetic acid (50 μM), N-glucose (20 mM), gluta-
mime (2 mM), HEPES (10 mM), penicillin (100 units/ml), and streptomycin (100 μg/ml) for a period of 1–3 h. Macrophage viability was checked at the end of the experiment with trypan blue dye exclusion and was found to be in the range of 92–96%.

Detection of NO—NO rapidly reacts with oxymyoglobin (Fe2+-O2) to form metmyoglobin (Fe2+–O2) and nitrate. Oxymyoglobin was prepared by reducing an approximately 500 μM solution of myoglobin (Sigma) in water with excess sodium dithionite (Fluka, Milwaukee, WI) and was desalted by passage through a Sephadex G-25 column (PD-10; Amer-
sham Pharmacia Biotech). The concentration of oxymyoglobin in the eluate was determined from the maximum at 582 nm with an extinction coefficient of 14,100 at 1 cm

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1 The abbreviations used are: NO, nitric oxide; NOS, nitric-oxide synthase; iNOS, inducible NOS; DAN, 2,3-diaminonapthalene; IL, interleukin; LPS, lipopolysaccharide; TNF, tumor necrosis factor; PAPA/NO, (Z)-1-(N-ammoniopropyl)-N-(n-propyl)amino[diazen-1-ium-1,2-diolate.

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Hewlett-Packard, Boulder, CO) and was adjusted to 10 µM in assay buffer. Metmyoglobin formation was determined from the change in absorbance at 400 and 420 nm (∆A400 49,000 M⁻¹ cm⁻¹ and ∆A420 51,000 M⁻¹ cm⁻¹; 20).

Electrochemical measurement of NO in assay buffer was performed with a Decalectrode (Amber, MI) electrode based on the design of Shibuki (21). The electrode was calibrated using a NO saturated solution as described previously (22). For these measurements, cells were cultured in 35-mm² Petri dishes (Falcon) at a density of 1 × 10⁶/ml in an incubator equipped to accommodate the probe assembly. Following stimulation with cytokines or LPS, the culture medium was replaced with the assay buffer immediately prior to analysis. The electrode was lowered with a mechanical manipulator to within 100 µm of the plate bottom.

Detection of Nitrite and Nitrate—The concentration of both nitrite (NO₂⁻) and nitrate (NO₃⁻) in the assay buffer was determined with a Seivers NO analyzer (Boulder, CO). Sample aliquots were injected into an anerobic (argon-purged) reaction vessel containing either sodium iodide/glacial acetic acid or vanadium chloride/hydrochloric acid (1 mM, 90 °C) to reduce either nitrite or (nitrate and) nitrate to NO, respectively. Subsequently, NO was drawn into the detector by vacuum and reacted with O₃. This chemiluminescent reaction was quantified and integrated with a photomultiplier tube/computer system. Nitrite values were validated using acidic Griess reagent and were found to correspond within a ±5% range.

Synthetic NO Donors—The 1-substituted diazen-1-ium-1,2-diolate NONOate compound PAPA/NO (23) was diluted from stock solutions in 10 mM NaOH. Concentrations were calculated from the absorbance at 250 nm (ε = 8,000 M⁻¹ cm⁻¹) with a spectrophotometer (8452A; Hewlett-Packard). Sodium nitroprusside was diluted directly into assay buffer.

Detection of Nitrosation—Formation of N₂O₃ was monitored indirectly by N-nitrosation of 2,3-diaminonaphthalene (DAN) (Sigma) in assay buffer, which yields the fluorescent product 2,3-naphthotriazolone (triazole) (24). Immediately prior to use, a 100 mM stock solution of DAN was prepared in dimethylformamide (Sigma) and then diluted in assay buffer to a final concentration of 200 µM. Aliquots (400 µM) of culture supernatant were diluted 1:4.75 in 10 mM NaOH. Fluorescence was measured on a Perkin-Elmer LS-50B fluorometer at an emission wavelength of 450 nm after excitation at 375 nm (5-nm slit widths).

Western Blot Analysis of iNOS—Macrophages were washed, harvested in cold 40 mM Tris solution with protease inhibitors, and then lysed by sonication. Proteins were denatured in a SDS reducing solution at 100 °C, separated by electrophoresis in a 4–12% Tris-glycine gel (Novex, San Diego, CA), and then transferred to a polyvinylidene difluoride membrane. iNOS was visualized as a 130-kDa band at 100 °C, separated by electrophoresis in a 4–12% Tris-glycine gel (Novex, San Diego, CA), and then transferred to a polyvinylidene difluoride membrane. iNOS was visualized as a 130-kDa band.

RESULTS

Nitrite, Metmyoglobin, and Nitrate Formation by ANA-1 Cells—A linear increase in the rate of nitrite production by ANA-1 macrophages was observed following stimulation for all conditions; however, these changes were dependent on prior activation with IFN-γ (data not shown). IFN-γ-primed ANA-1 cells subsequently treated with LPS produced 2.5-fold more nitrite, Metmyoglobin, and Nitrate Formation by ANA-1 Cells—A linear increase in the rate of nitrite production by ANA-1 macrophages was observed following stimulation for all conditions; however, these changes were dependent on prior activation with IFN-γ (data not shown). IFN-γ-primed ANA-1 cells subsequently treated with LPS produced 2.5-fold more nitrite than those treated with either TNF-α or IL-1β (Fig. 1A). However, the absolute level of nitrite exceeded metmyoglobin formation by 20–30%.

In contrast to nitrite formation, a low level of nitrate production (2 nM/min) was observed independent of stimulation status. Substitution of l-arginine in the assay buffer with N-methylarginine reduced the rate of nitrite production by ANA-1 cells to 0.5 nM/min for all treatment conditions but did not affect nitrate levels.

iNOS Protein—A single 130-kDa band corresponding to iNOS protein was observed on Western blots of lysates from activated ANA-1 cells (Fig. 1C). Treatment with IFN-γ followed by either TNF-α or IL-1β induced a 38–41% increase in the absorbance from background levels observed with unstimulated cells. The IFN-γ/LPS stimulant combination resulted in an 86% increase. Although these data are only qualitative, the relative differences between stimulant conditions for iNOS protein levels (2-fold) were similar to the differences observed with nitrite and oxymyoglobin oxidation.

Detection of NO by Electrode and Nitrosative Capacity—The relative amount of NO released into the assay buffer by ANA-1 cells was assessed with an electrochemical probe (Fig. 2A). A slight increase over baseline values was observed with IFN-γ-primed cells following stimulation with either TNF-α or IL-1β. In contrast, a substantial increase in NO concentration was evident following the treatment of IFN-γ-primed cells with LPS.

Low levels of triazole (0.09–0.15 mM/min), formed by the nitrosation of DAN in the assay buffer, were detected in the supernatant of cells treated with either IFN-γ/L-1β or IFN-γ/TNF-α (Fig. 2B). Nitrosoation of DAN by cytokine-stimulated cells was not observed when the assay buffer containing less than 1 mM l-arginine. Markedly higher levels of triazole formation (3.83 mM/min) was observed in cultures treated with the IFN-γ/LPS stimulant combination. DAN nitrosation by LPS-stimulated cells was reduced to 0.1 mM/min when the concentration of l-arginine was reduced to 5 µM. No triazole formation was observed when DAN (200 µM) was added to (DAN-free) culture supernatant at the end of the 3-h assay period.

Data were collected from cells stimulated with IFN-γ/LPS plated at densities ranging from 4 × 10⁵ (nearly confluent) to...
0.1 × 10^5 cells/ml to evaluate nitrosative capacity as a function of nitrite production (Fig. 3). Approximately 1.8 × 10^5 IFN-γ/LPS cells produced 20 nM nitrite/min, which was the level equal to that produced by 4 × 10^5 cytokine-stimulated cells (IFN-γ and either IL-1β or TNF-α; see Fig. 2A). At this point of equivalence for nitrite, the rate of triazole formation by cells stimulated with IFN-γ/LPS was 1.0 nM/min, whereas cells activated with only cytokines was 0.1 nM/min.

Intact and Lysed Cells—To determine the significance cellular integrity on the activity of iNOS, either cytokine- or LPS-activated ANA-1 cells were dislodged from Petri dishes by scraping and were suspended in 1 ml of assay buffer containing optimal levels of tetrahydrobiopterin, FAD, FMN, NADPH, and protease inhibitors at a density of 1 × 10^6 cells. Lysis of IFN-γ/TNF-α-stimulated cells with by a brief sonication pulse resulted in a 10% decrease in the rate of nitrite production relative to that of intact cells. Lysis of IFN-γ/LPS-stimulated cells resulted in a 49% reduction in nitrite production. The nitrosation of DAN in these preparations could not be evaluated due to spectral interference from the iNOS cofactors.

Scavenger Profiles—To discriminate between the various reactive nitrogen oxide intermediates produced by ANA-1 macrophages that may be responsible for either promoting or quenching the nitrosation of DAN in the media, a panel of scavenger compounds was tested (Table I). Catalase, Cu/Zn-superoxide dismutase, and uric acid were added to the assay buffer to assess the status of hydrogen peroxide, superoxide, and peroxynitrite, respectively (and radicals generated by Haber-Weiss reactions). The presence of these scavenger components did not have an effect on the levels of either nitrite, DAN nitrosation, or dihydrorhodamine oxidation (data not shown) under all stimulant conditions. The iron chelators diethylenetriamine pentaacetic acid and 2,3-bipyridine also had no effect on the ability of ANA-1 cells stimulated with IFN-γ and LPS to either form nitrite or nitrosate DAN. However, 1,10-phenanthroline at high concentrations (IC_50 = 5750 μM) was able to affect triazole formation. The addition of N_2O_3 scavengers azide, ascorbic acid, or glutathione to the assay buffer quenched DAN nitrosation by ANA-1 cells stimulated with IFN-γ and LPS in a dose-dependent manner without reducing nitrite formation. Compounds that alter the cellular redox balance (e.g. deferoxamine and buthionine sulfoximine) added concomitantly with either cytokines or LPS reduced both nitrite and DAN nitrosation approximately 50%. A similar effect was ob-

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served when either FCS or essential amino acids were omitted from the culture media.

Comparison of Profiles from Cellular and the Synthetic NO Sources—The synthetic NO donor PAPA/NO generates a sustained high flux of NO in assay buffer with a half-life of 20 min (23). Nitrate formation from PAPA/NO was not observed. Approximately 5 μM PAPA/NO produced both nitrite and nitrosated DAN at rates equivalent to 4 × 10^5 adherent ANA-1 cells stimulated with IFN-γ/LPS under identical conditions (e.g. 1 ml of assay buffer in a 24-well plate at 37 °C and 5% CO2, 95% air; Fig. 4A and B). In contrast, a 225 μM concentration of the iron-nitrosyl (NO^+ donor) compound sodium nitroprusside was required to nitrosate DAN at a rate equivalent to (IFN-γ/LPS) ANA-1 cells (Fig. 4). The DAN nitrosation IC_{50} values of ascorbic acid and glutathione were similar for IFN-γ/LPS-stimulated ANA-1 cells and 5 μM PAPA/NO (Fig. 5).

**DISCUSSION**

Synthetic donors such as PAPA/NO can generate NO flux sufficient to elicit nitrosation reactions via N\textsubscript{2}O\textsubscript{3}. Of the NOS isozymes, only iNOS can catalyze the formation of similarly high levels of NO for prolonged periods of time. This is due, in part, to the continuous association of calmodulin with iNOS and a decreased susceptibility to product feedback inhibition. The present study showed that the capacity of cells to nitrosate extracellular target compounds was not due solely to the presence of iNOS but was also dependent on the nature of the inductive stimuli.

**Relationship between Nitrates, Nitrate, and NO Trapped by Oxymyoglobin**—Nitrates and nitrate are stable higher oxidation products of NO that are often used as indirect measurements of NOS activity in aerobic aqueous media (25, 26). Nitrate can be derived from numerous nitrogen oxide reactions. The stimulation of IFN-γ-primed ANA-1 cells with bacterial LPS resulted in the formation of approximately 2-fold more nitrate than that formed by cells stimulated with the cytokines IL-1β or TNF-α (Fig. 1A). For each condition, nitrate values were highly correlated with the level of NO trapped by extracellular oxymyoglobin (Fig. 1B); however, the rate of nitrate production was greater than that of oxymyoglobin oxidation. NO is trapped extremely rapidly by the dioxygen ferrous heme adduct of oxymyoglobin (k = 3.4 × 10^7 s^-1 M^-1; Ref. 27). These data suggest that a portion of the NO derived from iNOS was oxidized to nitrite prior to leaving the cell and that this occurs regardless of the immunostimulant combinations used to induce iNOS expression.

Nitrate is derived primarily from the interactions of NO with oxyhemoproteins. An apparent constitutive release of nitrate (2 mM/min) from unstimulated ANA-1 cells was observed. Substitution of L-arginine with the iNOS competitive inhibitor N\textsubscript{N}-methylarginine, however, showed that this nitrate was not derived from endogenously produced NO. These data suggest that a modest portion of nitrate was taken up from the culture media via anion transporters and was subsequently released following incubation in nitrate-free buffer during the assay period (25). A minor level of iNOS-specific nitrate (2 mM/min) production was distinguished from background only in cultures of ANA-1 cells stimulated with IFN-γ and LPS.

**iNOS Inductive Signals Confer High NO Level and Nitrosative Capacity**—The concentration of NO derived from ANA-1 cells was measured either indirectly by oxymyoglobin oxidation or directly with an electrochemical probe. The oxymyoglobin data show that IFN-γ-primed ANA-1 cells produced incrementally greater amounts of NO following stimulation with either TNF-α, IL-1β, or LPS. In contrast, an electrochemical probe placed within 100 μM of the cell bed detected a substantial amount of NO in the assay buffer only with the IFN-γ/LPS stimulant combination (Fig. 2A). Whereas oxymyoglobin can be envisioned as millions of randomly distributed highly sensitive NO probes, the electrochemical probe registers the presence of NO at a fixed and more distal point in space.

The stark contrast in NO profiles with the electrochemical probe predicts that the nature of the NO interactions with molecular targets in the milieu surrounding a macrophage will differ dependent on the signals received for iNOS induction. This dichotomy was clearly illustrated in the capacity of murine macrophages to nitrosate the extracellular target compound DAN. Consistent with an earlier report using RAW 264.7 cells (15), IFN-γ-primed ANA-1 cells stimulated with LPS nitrosated DAN present in the media. In comparison, the rate of DAN nitrosation in sister cultures of IFN-γ-primed ANA-1 cells stimulated with either TNF-α or IL-1β was 85–90% lower (Fig. 2B). This dichotomy was also observed with primary murine macrophages, the IC-3, J774, and RAW 264.7 cell lines (data not shown). In addition, IFN-γ-primed macrophages nitrosated DAN following stimulation with LPS derived from Gram-positive bacteria as well as viral and parasite antigens (data not shown).

**Relationship of Nitrosative Capacity to iNOS Levels and Specific Activity**—The addition of superoxide dismutase, catalase, and urate to the assay buffer did not affect the levels of NO formation under each stimulant condition (Table I). These data suggested that the dichotomy for DAN nitrosation were not related to the differential consumption of NO by oxidative pathways. Rather, the mechanisms may operate at both the transcripational and posttranscriptional levels. Western blot analysis showed that ANA-1 macrophages stimulated with IFN-γ and LPS had more than twice the iNOS protein of those treated with cytokines (Fig. 1C). These data correspond to the differences observed for both nitrite production and oxymyoglobin oxidation. Nitrite production and nitrosative capacity were plotted as a function of cell density to examine whether nitro-
Satisfactory capacity was determined by the amount of iNOS per cell and the subsequent mass transport of NO. At an equivalence for nitrite production (and by extension, iNOS), the nitrosative capacity of ANA-1 cells stimulated with IFN-γ/LPS exceeded that of those stimulated with cytokines by 8–10-fold (Fig. 3). These data show that the specific activity of iNOS for nitrite formation and cellular nitrosative capacity are not coupled. Differences in nitrosative capacity may be related to the physical configuration of iNOS within the cell. Incubation of a sonication lysate derived from IFN-γ/LPS-stimulated ANA-1 cells in an optimized reaction mixture resulted in a 50% reduction in nitrite formation relative to that produced by intact cells. In contrast, lysis of ANA-1 cells stimulated with IFN-γ and TNF-α resulted in only a 10% reduction in nitrite formation. These data rule out the significance of arginine transport and the availability of iNOS co-factors (e.g. NADPH, tetrahydrobiopterin, and flavins) in conferring nitrosative capacity. Dilution of the cell contents upon lysis may alter the ratio between dimeric and inactive monomeric forms of iNOS in a manner independent of tetrahydrobiopterin levels (29, 30). Alternatively, a disproportional decrease in nitrite production from lysed IFN-γ/LPS-stimulated ANA-1 cells may reflect the disruption of a membrane association critical for catalytic activity. Although metabolic incorporation of radiolabeled myristate, palmitate, and acetate onto iNOS was not observed in immunoprecipitates from IFN-γ-stimulated macrophages (31), the status of iNOS acylation or prenylation in macrophages further stimulated with either cytokines or LPS remains unclear.

Characterization of the Nitrosating Species—Synthetic iron
nitroso complexes can catalyze NO exchange reactions through what has been termed transnitrosation (18, 32). The elegant work by Lancaster and co-workers (16, 17) has shown that cells such as macrophages and hepatocytes form iron nitroso complexes following iNOS induction with cytokines and LPS. A potential role for iron nitroso complexes in the nitrosation of DAN, however, was not supported by several data. First, the addition of iron scavengers to the assay buffer did not impede DAN nitrosation by IFN-γ/LPS-stimulated ANA-1 cells (Table 1). An interesting exception, albeit at high concentrations, was phenanthroline. Second, approximately 225 μM (70 ng/μl) of the prototypical iron nitroso (NO⁺ donor) compound sodium nitroprusside was required to match the rate of DAN nitrosation observed with 4 × 10⁵ IFN-γ/LPS-stimulated ANA-1 cells (Fig. 5A). S-Nitrosation of albumin by synthetic dinitrosyl iron complexes also required concentrations in the high micromolar range (18). A recent study found that the cumulative effect of IFN-γ/LPS treatment on J774 macrophages was to decrease iron uptake (to less than 2 pg per 4 × 10⁵ cells) rather than increase iron release (33). Taken together, these data suggest that the observed level of DAN nitrosation by ANA-1 cells in our 3-h assay period was not due to an iron nitroso complex mechanism.

Nitrite production was correlated with the NO profiles observed using oxyhemoglobin regardless of stimulant status. In contrast, triazole formation from DAN and electrochemical detection of NO were exclusive to macrophages induced with IFN-γ/LPS. An enhanced capacity for NO to travel from a source cell to a distal point alters the potential reactivity of NO at that point. Specifically, the formation of the nitrosating agent N₂O₃ may predominate under these conditions. N₂O₃ was not initially accepted as a relevant species in biological systems because the rate constant for the reaction between NO and O₂ was considered to be too slow (k = 2 × 10⁶ M⁻¹ s⁻¹; Ref. 34) to compete with other potential pathways for NO consumption (e.g. hemeproteins and superoxide). However, the rate of the NO/O₂ reaction has a second-order dependence on NO concentration, indicating that the formation of N₂O₃ can prevail when the flux of NO is high (34–36).

The synthetic NO donor PAPA/NO generates N₂O₃ in an aerobic environment. Formation of both nitrite and triazole by 4 × 10⁵ ANA-1 cells stimulated with IFN-γ/LPS was equivalent to that generated by approximately 5 μM PAPA/NO (Fig. 4). For both ANA-1 cells and PAPA/NO under these conditions, the IC₅₀ values for the N₂O₃ scavenger compounds ascorbic acid and glutathione were remarkably similar (Fig. 5). These data suggest that N₂O₃ derived from the NO/O₂ reaction was the nitrosating agent for DAN consistent with the previous findings of Tannenbaum and co-workers (14). The partitioning of NO and O₂ to the hydrophobic regions of membranes can greatly accelerate their reactivity with each other (37). However, two observations suggest that N₂O₃ was formed outside the cell. First, the profiles for NO in the media using the electrochemical probe and the nitrosation of DAN are congruous. Second, the uptake mobilities of ascorbate and glutathione into the macrophage cytoplasm are very different (14). However, the quenching profiles of both ascorbate and glutathione were similar between nitrosating cells and PAPA/NO in solution.

Conclusion—This study shows that nitrosative alterations may occur to biomolecules containing either thiol or amino moieties in cells neighboring macrophages only if iNOS was induced in a selective manner (e.g. activation by direct contact with pathogens or their products). Nitrosative conditions generated by synthetic NO donors have been shown to inhibit the activity of enzymes involved in glycolysis (37) and DNA repair (38, 39). The nitrosative capacity of 4 × 10⁵ LPS-activated ANA-1 cells was equivalent to that of 5 μM PAPA/NO in a volume of 1 ml (Fig. 4B). In the much smaller dimensions of a biological compartment, macrophages may elicit nitrosative stress through N₂O₃-mediated reactions equivalent to higher concentrations of NO donor. We have observed that IFN-γ/LPS-stimulated macrophages can inactivate target enzymes in neighboring cells equivalent to that achieved with millimolar levels of synthetic NO donor.²

Despite many advances in our understanding of redox mechanisms, the role of nitrosative stress in disease processes is poorly understood. This study provides a novel framework for deciphering how macrophages may regulate the nitrogen oxide chemistry derived from iNOS during specific pathological situations. The data show that macrophage exposure to inflammatory cytokines in the absence of direct contact with infectious agents or their products was insufficient to prompt a nitrosative profile. Determination of the mechanisms that govern the relationship between nitrosative and oxidative stress will unlock many fundamental aspects regarding how nitrogen oxides are involved in fighting pathogens (40), regulating cell-mediated immunity (41, 42), and eliciting deleterious bystander effects on host tissue (7–9). Identification of genes and proteins that act as nitrosative switches may provide new pharmacological targets to control iNOS-generated nitrogen oxide chemistry in a wide range of diseases.

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REFERENCES


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Macrophage-mediated Nitrosative Stress
