ORIGINAL ARTICLES

Effect of nitric oxide on megakaryocyte growth induced by thrombopoietin

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The present study investigated the effect of nitric oxide (NO) on megakaryocyte (Mk) proliferation induced by thrombopoietin (TPO). Low-density mononuclear cells (MNCs) and CD34+ cells from human bone marrow (BM) were cultured in liquid medium in the presence of sodium nitroprusside (SNP) or (Z)-1-(2-(aminoethyl)-N-(2-ammonioethyl) amino) diazen-1-ium-1, 2-diolate (DETA/NO) and then stimulated with TPO. Mk number decreased in both NO donors, as identified by flow cytometry 11 to 13 days after TPO stimulation. Nitrite, cyanide, or the carrier molecule DETA failed to reproduce the inhibition caused by NO donors. When CD34+ cells were treated with DETA/NO, the inhibition of Mk growth was even more pronounced than that in MNCs. Failure of the guanosine 3′,5′-cyclic monophosphate (cGMP) analog 8-bromoguanosine 3′,5′-cyclic monophosphate (8-Br-cGMP) to inhibit Mk proliferation suggests that cGMP is not involved in Mk suppression mediated by NO. On the other hand, DNA analysis by flow cytometry showed that apoptosis of CD34+ cells and Mks seemed to be at least one of the mechanisms associated with the cytotoxic DETA/NO effect. Stimulation of MNCs or CD34+ cells with tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) increased endogenous NO levels and suppressed Mk growth. Treatment with NO synthesis inhibitors such as L-N^G-monomethyl arginine (L-NMMA) or L-N^G-nitroarginine methyl ester hydrochloride (L-NAME) partially reversed Mk growth inhibition induced by TNF-α and IFN-γ, although increased NO levels returned to normal values. The results presented here strongly indicate that NO regulates the growth of Mks induced by TPO by a direct effect on both progenitors and mature Mks. (J Lab Clin Med 2001;137:261-9)

Abbreviations: 8-Br-cGMP = 8-bromoguanosine 3′,5′-cyclic monophosphate; BM = bone marrow; cAMP = adenosine 3′,5′-cyclic monophosphate; cGMP = guanosine 3′,5′-cyclic monophosphate; DETA/NO = (Z)-1-(2-(aminoethyl)-N-(2-ammonioethyl) amino) diazen-1-ium-1, 2-diolate; DNaseI = deoxyribonuclease I; FITC = fluorescein isothiocyanate; IFN-γ = interferon-γ; IL-3 = interleukin-3; IMDM = Iscove modified Dulbecco's medium; L-arg = L-arginine; L-NAME = L-N^G-nitroarginine methyl ester hydrochloride; L-NMMA = L-N^G-monomethyl arginine; Mk = megakaryocyte; Mkp = megakaryocytopoiesis; MNC = low-density mononuclear cell; NADPH = reduced nicotinamide adenine dinucleotide phosphate; NO = nitric oxide; NOS = nitric oxide synthetase; PI = propidium iodide; SNP = sodium nitroprusside; TNF-α = tumor necrosis factor-α; TPO = thrombopoietin

Fig 1. Inhibition of Mk growth by DETA/NO. MNCs (7.5×10^5) were treated with different concentrations of DETA/NO before TPO stimulation. The absolute number of Mks was determined at days 11 through 13. Values are expressed as mean ± SEM of six independent experiments. The *asterisk* indicates statistically significant differences ($P < .05$) between treatments and controls.

Nitric oxide is a free radical endogenously synthesized from L-arg by at least three isoenzymes. Two of them are constitutive and synthesize NO, known to physiologically regulate vascular tone, platelet aggregation, and neuron-neuron interaction.[1] The third enzyme is induced by proinflammatory cytokines and produces great amounts of NO, transforming NO from a physiologic mediator into a cytostatic and cytotoxic molecule.[1] Whereas most physiologic effects of NO are mediated by the activation of a soluble guanylate cyclase,[2] cytotoxic effects include other targets such as binding to iron in the prosthetic groups of proteins, nitrosylation of proteins, inhibition of ribonucleotide reductase, and inhibition of diverse enzymes of the respiratory chain.[3-6]

NO has also been shown to regulate the growth of many cell types, including hematopoietic cells.[7-9] Maciejewski et al[10] documented that NO inhibits the growth of erythroid and myeloid colonies, while Shami et al[11] found a selective effect of NO on colony formation depending on both its concentration and whether mononuclear or CD34+ cells were used.

Among the hematopoietic processes, the regulation of Mkp has been less extensively studied because of the lack of a specific growth factor for Mks. During 1994, five research groups characterized TPO as the primary regulator of Mk development and platelet production in vitro and in vivo.[12-16]

The precise molecular mechanisms involved in the signal-transduction pathway between TPO and its receptor, c-mpl, have not yet been completely discerned. Binding of TPO to its receptor results in the dimerization of c-mpl and the activation of members of the Janus family of kinases and of signal-transducing

proteins.[17] Little is known regarding the role of second messengers such as cGMP or cAMP in the development of Mks. Before the cloning of TPO, it had been demonstrated that exposure of Dami cells to cAMP analogs or to agents raising intracellular cAMP levels inhibited cell growth.[18] Differential effects were observed in Mk spreading and pro-platelet formation. Although elevated intracellular cAMP inhibited Mk spreading, pro-platelet structures were stimulated.[19]

The regulatory role of NO on Mkp has not yet been investigated. Because NO is produced by most human BM cells,[20-22] in the present study we investigated the effect of NO on human Mk growth induced by TPO.

METHODS

Preparation of MNCs. BM samples were collected in accordance with the guidelines of the Institutional Ethical Committee on Human Subjects. BM, obtained from the femur of hematologically normal patients having total hip arthroplasty, was collected in a special anticoagulant mixture containing 50 U/mL preservative-free heparin (Sigma, St Louis, MO), 1 mmol/L ethylenediaminetetraacetic acid disodium salt, and 0.1 mg/mL DNaseI (Boehringer Mannheim, Mannheim, Germany). Marrow cells were repeatedly extracted from bone fragments with DNaseI, and MNCs were isolated by a Ficoll-Paque gradient as previously described.[23] Residual red cells were lysed with NH_4Cl, and remaining cells were recovered by centrifugation through a 10% human serum albumin cushion. For CD34+ cell purification, adherent cells were discarded after overnight incubation in thioglycerol-free IMDM (GIBCO, Grand Island, NY) containing 10% fetal bovine serum (GIBCO) and nonessential amino acids (0.1 mmol/L)(GIBCO). All culture media were supplemented with 100 U/mL penicillin and 100 μg/mL streptomycin, and incubation was carried out at 37°C in a 5% CO_2 fully humidified atmosphere.

Purification of CD34+ cells. CD34+ cells were purified from non-adherent MNCs by positive selection with the CD34 magnetic cell sorting Mini-MACS kit (Myltenyi Biotec, Auburn, CA) in accordance with the manufacturer's recommendations. The purity of the CD34+-enriched population was determined by immunolabeling the cells with a monoclonal antibody, anti-CD34 FITC-conjugated (Immunotech-Amac, Westbrook, ME), that reacted with an epitope other than the antibody used for separation. After two Mini-MACS column separations, cells were 90% to 95% CD34+.

Culture conditions. MNCs (7.5×10^5) or purified CD34+ cells (5×10^4) were cultured in liquid medium for 11 to 13 days at 37°C in a 5% CO_2 fully humidified atmosphere. Cells were grown in an IMDM-based medium supplemented with 1% human serum albumin, 0.1 mmol/L nonessential amino acids, 2 mmol/L L-glutamine, and 10% human serum obtained by recalcification of citrated platelet-free plasma. After culture, cells were counted with a hemocytometer with Trypan blue dye uptake to identify dead cells. After washing, cells were stained with FITC-conjugated anti-CD41 (glycoprotein IIb; Immunotech-Amac, Westbrook, ME), and the relative frequency of mature Mks was determined by flow
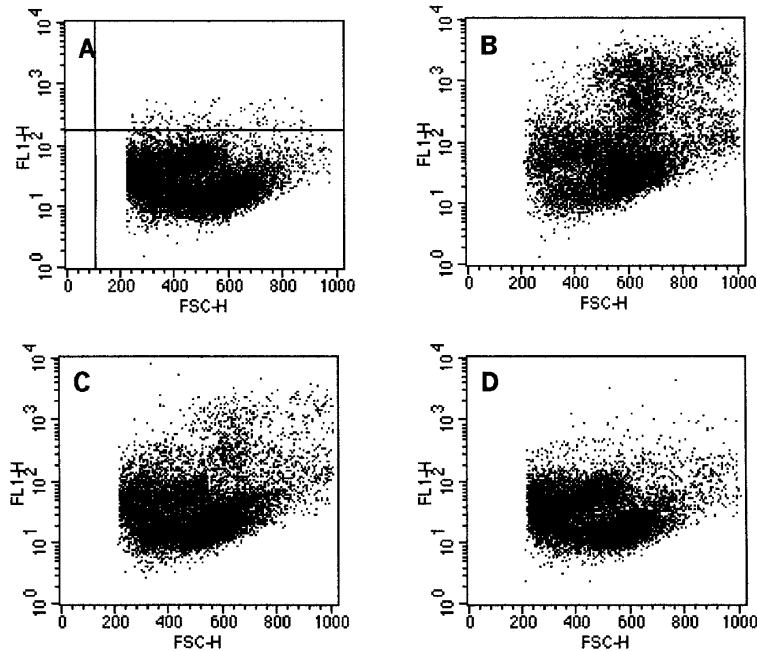
Fig 2. Inhibition of Mk growth by DETA/NO. MNCs (7.5×10^5) were stimulated with TPO (10 ng/mL) in the absence of DETA/NO (**B**), in the presence of 250 μmol/L DETA/NO (**C**), or in the presence of 500 μmol/L DETA/NO (**D**). After 12 days of culture, cells were stained with anti-CD41a (**B, C, D**) or with an equivalent concentration of an irrelevant isotypic monoclonal antibody (**A**). Shown is one representative experiment of six similar experiments.

cytometry. Negative controls were cells stained with an irrelevant FITC-anti-mouse IgG1 used at equivalent IgG1 concentrations.

Growth factor, cytokine, and NO inhibitor treatment. TPO and IL-3 (R&D Systems, Minneapolis, MN) were used at a concentration of 10 ng/mL. TNF-α and IFN-γ (Calbiochem, San Diego, CA) were used at 10 ng/mL and 100 U/mL, respectively. Fresh solutions of DETA/NO (Cayman), SNP (Sigma), L-NMMA (Calbiochem), and L-NAME (Sigma) were prepared immediately before use.

Flow cytometry analysis. Flow cytometry analysis was performed with a Becton Dickinson flow cytometer. Fluorescence attributable to FITC antibodies was excited by an argon laser operating at 488 nm. Emission from fluorescein was measured with bandpass filters of 525 nm. The percentage of positive cells was corrected by substracting the percentage of positive cells in the isotype stained control within the same integration region.

Nitrite/nitrate analysis. MNCs were seeded into 96-well microtiter plates at densities of 1×10^6 in 200 μL of culture medium. After 72 hours, cell-free supernatants were collected, centrifuged, and stored at –70°C until analysis. NO production was measured by nitrite/nitrate accumulation with a commercial kit (Calbiochem). In brief, nitrate was converted to nitrite by incubation with nitrate reductase in the presence of NADPH. Lactate dehydrogenase was then used to destroy excess NADPH. Equal volumes of sample and Griess reagent were incubated at room temperature. After 10 minutes, absorbance was read at 550 nm. The nitrite concentration was determined by using sodium nitrate as a standard.

Flow cytometry analysis for apoptosis. Apoptosis was determined by means of DNA histograms with flow cytometry analysis to identify hypodiploid nuclei. In brief, cells were washed, pelleted, and fixed in cold 70% ethanol. After 30 minutes of incubation at 4°C, cells were washed and resuspended in 500 μL PI solution (20 μg/mL; Sigma) containing ribonuclease A (10 μg/mL; Sigma). After 30 minutes of incubation, cells were analyzed by flow cytometry, and 10,000 events were counted. PI fluorescence of individual nuclei was recorded with the FL-2 detector. When apoptosis was evaluated in Mk-enriched suspensions, cells were labeled with anti CD41-FITC before PI staining, and the presence of hypodiploid cells was analyzed in the CD41+ population.
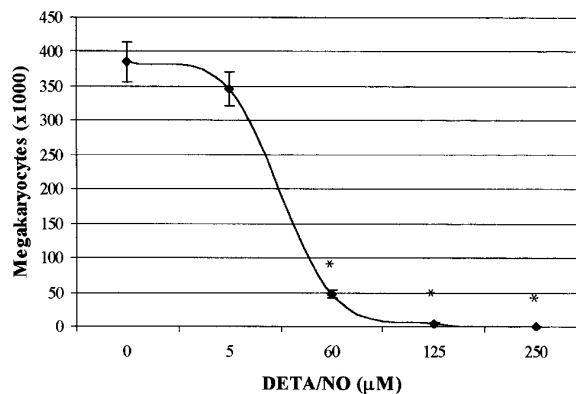
Statistical analysis. Results are expressed as mean ± SEM. Differences were evaluated by using the paired Student *t* test, and a value of $P < .05$ was considered statistically significant.

RESULTS

Inhibition of Mk growth by DETA/NO. The addition of DETA/NO to MNC cultures treated with TPO (10 ng/mL) resulted in marked inhibition of Mk production (Figs 1 and 2). The inhibitory effect of the NO donor was dependent on the concentration of the DETA/NO used, and it correlated not only with the decrease in total cell number but also with the reduction in CD41+ cell percentage (Table I). The effective threshold concentration was 125 μmol/L, and 500 μmol/L abrogated Mk formation.
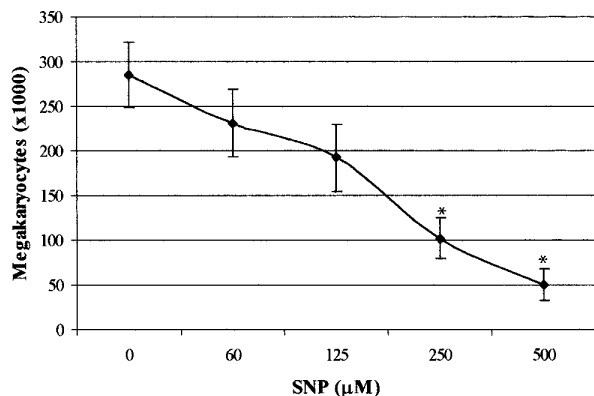
Fig 3. Inhibition of Mk growth by SNP. MNCs (7.5×10^5) were treated with different concentrations of SNP before stimulation with TPO (10 ng/mL). The absolute number of Mks was determined at days 11 through 13. Values are expressed as mean ± SEM of four independent experiments. The *asterisk* indicates statistically significant differences ($P < .05$) between treatments and controls.

Fig 4. Inhibition of Mk growth by SNP on MNC cultures stimulated with IL-3. MNCs (7.5×10^5) were stimulated with IL-3 (10 ng/mL), and different concentrations of SNP were added before IL-3. The absolute number of Mks was determined at days 11 through 13. Values are expressed as mean ± SEM of four independent experiments. The *asterisk* indicates statistically significant differences ($P < .05$) between treatments and controls.

Fig 5. Effect of DETA/NO on Mks derived from CD34+ cells. CD34+ cells (5×10^4) were treated with indicated concentrations of DETA/NO before stimulation with TPO (10 ng/mL). The absolute number of Mks was determined at days 11 through 13. Values are expressed as mean ± SEM of five independent experiments. The *asterisk* indicates statistically significant differences ($P < .05$) between treatments and controls.

Table I. Effect of DETA/NO on total cell number and percentage of CD41+ cells in MNC cultures stimulated with thrombopoietin

DETA/NO (μmol/L)	Total cell number ($\times$ 103)	CD 41+ cells (%)
0	1048 ± 65	25 ± 4
5	955 ± 145	25 ± 5
60	893 ± 140	23 ± 4
125	660 ± 116*	17 ± 3*
250	487 ± 165*	13 ± 2*
500	302 ± 130*	7 ± 5*

Values represent the mean ± SEM of six independent experiments. The *asterisk* indicates statistically significant differences ($P < .05$) between treatments and controls.

To verify whether the inhibitory effect of DETA/NO was related to the presence of the NO moiety rather than to the carrier molecule or to the released nitrite, we investigated the effect of DETA and nitrite. When MNC cultures were treated with DETA (250 μmol/L) or nitrite (250 μmol/L), Mk proliferation was not statistically different from that in control cultures (207 ± 32 $\times$ 10³ Mks, 232 ± 30 $\times$ 10³ Mks, 240 ± 20 $\times$ 10³ Mks, respectively, n = 3).

Inhibition of Mk growth by SNP. To further demonstrate that Mk growth suppression was attributable to NO, the effect of SNP, a second NO-donating agent, was used. It is known that SNP releases NO in seconds,[24] in con-

trast to DETA/NO, which releases NO with a half-life of 20 hours at pH 7.4.[25] Fig 3 shows that the addition of SNP, like the addition of DETA/NO, at day 0 of initiating MNC culture in the presence of TPO, also inhibited Mk formation in a concentration-dependent manner. The inhibitory effect was not caused by the ferricyanide moiety generated by SNP, because the number of Mks produced by MNC cultures treated with potassium ferricyanide (125, 250, and 500 μmol/L) was not statistically different from control values (258 ± 15 $\times$ 10³ Mks, 262 ± 8 $\times$ 10³ Mks, 251 ± 10 $\times$ 10³ Mks, and 233 ± 10 $\times$ 10³ Mks, respectively; n = 3).

In an attempt to investigate whether inhibition mediated by NO was related to a specific mechanism of TPO-induced Mk growth, MNCs were stimulated by IL-3 in the presence of SNP. After 12 days of culture, Mk development was significantly suppressed in cultures treated with SNP as compared with control samples (Fig 4).
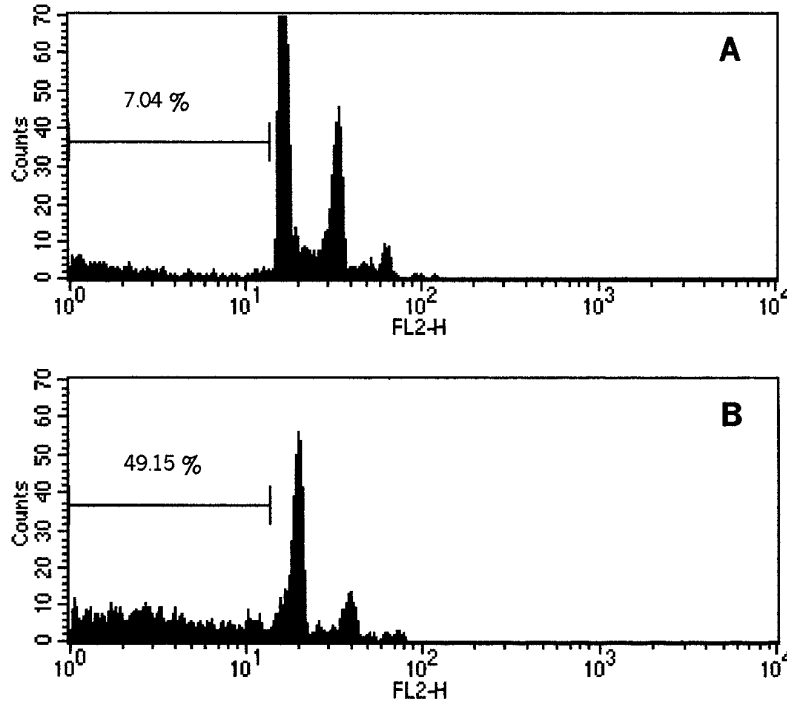
Fig 6. Flow cytometry analysis of apoptosis induced by DETA/NO. CD34^{+} cells untreated (**A**) or treated (**B**) with DETA/NO (125 μmol/L) were stimulated with TPO. After 96 hours, cells were stained with PI and analyzed for red fluorescence emission. One representative experiment of four similar experiments.

Effect of DETA/NO on Mk growth derived from CD34^{+} cells. To determine whether hematopoietic progenitors or accessory cells were affected by NO, we evaluated the effect of DETA/NO on an enriched population of CD34^{+} cells (Fig 5). DETA/NO not only blocked formation but it was also more potent than in MNC cultures, since even at 60 μmol/L of DETA/NO there was significant inhibition of Mk growth (>80%). This concentration of DETA/NO was ineffective on Mk differentiation from MNCs.

Because an increase in cGMP is one of the major intracellular mediators involved in the NO signaling pathway, we tested the effect of the soluble and hydrolysis-resistant analog of cGMP, 8-Br-cGMP. None of the employed concentrations of 8-Br-cGMP exerted any inhibitory effect on Mk growth as compared with control cultures ($398 \pm 98 \times 10^3$ Mks, $438 \pm 109 \times 10^3$ Mks, and $420 \pm 70 \times 10^3$ Mks at 0, 3, and 6 mmol/L, respectively; n = 3).

Effect of DETA/NO addition at different days after stimulation of CD34^{+} cells with TPO. To investigate whether only CD34^{+} cells or Mk progenitors were sensitive to the NO cytotoxic effect, DETA/NO was added at different time points after CD34^{+} cell differentiation was initiated by TPO. Under our experimental conditions, 8 days after stimulation of TPO, $72\% \pm 5\%$ of cells

**Table II.** Effect of DETA/NO addition on Mk growth after different times after TPO stimulation

Culture day of DETA/NO addition	Mks (%) of control	Cellular viability (%)
1-7	<20	<20
8	50 ± 10	30 ± 6
9	100	42 ± 5
10	100	60 ± 8

DETA/NO (125 μmol/L) was added at indicated days after TPO stimulation of CD34+ cells. Absolute number of Mks and cell viability by exclusion of trypan blue was determined at day 11. Values represent the mean ± SEM of three independent experiments.

were CD41^{+}, and their proliferation ceased (n = 3).

The addition of DETA/NO (125 μmol/L) from day 1 to day 7 had a marked cytotoxic effect, since hardly any cells were obtained at the end of culture (day 11, n = 4). When DETA/NO was added at day 8 or day 10 after stimulation with TPO, significantly more cells were obtained, but cell viability decreased to 60% even as early as 24 hours after the addition of the NO donor (Table II).

DETA/NO-induced apoptosis of CD34^{+} cells and Mks. CD34^{+} cells, MNCs, or Mk-enriched suspensions treat-
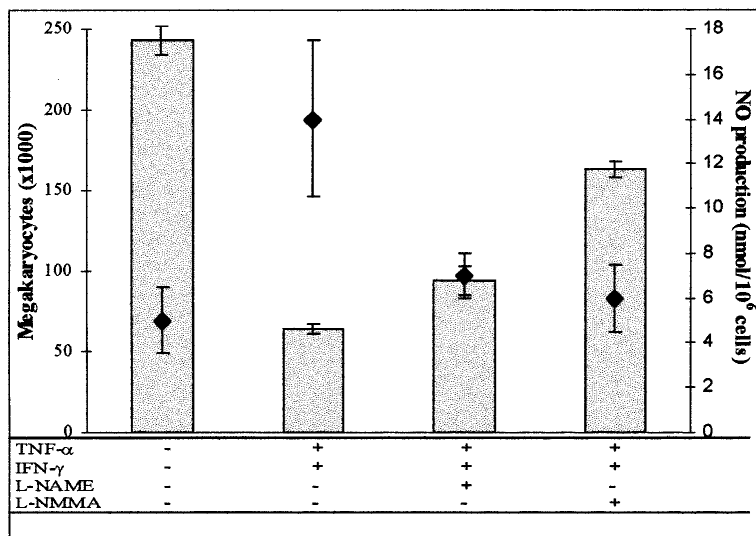
Fig 7. Effects of TNF-α and IFN-γ on Mk proliferation and NO production in MNCs. MNCs were treated with TNF-α and IFN-γ with or without L-NMMA (0.5 μmol/L) or L-NAME (0.5 μmol/L). The absolute number of Mks (*bars*) and NO production (◆) in MNC supernatants was determined at days 11 through 13 days or 72 hours after TPO stimulation, respectively. Values represent the mean ± SEM of four independent experiments.

Table III. Percentage of apoptosis of CD34+ cells and Mks induced by DETA/NO

Hours after DETA/NO	CD34+		Mks	
	Control	DETA/NO	Control	DETA/NO
24	4 ± 0.8	12 ± 1.6	7 ± 0.4	36 ± 3
48	6 ± 0.8	30 ± 3	10 ± 0.8	54 ± 5
72	6 ± 0.4	34 ± 4	11 ± 0.8	69 ± 2
96	7 ± 0.3	45 ± 4	16 ± 0.4	67 ± 8

CD34+ cells or Mks (derived from CD34+ cells stimulated for 8 days with TPO) were treated with DETA/NO in the presence of TPO. At indicated times after DETA/NO addition, the presence of hypodiploid cells was examined by flow cytometry. Values are expressed as mean ± SEM for three different experiments.

ed with DETA/NO presented a higher percentage of hypodiploid cells as compared with non-treated samples (Fig 6). The apoptotic effect of DETA/NO was greater in Mks than in CD34+ cells, although the percentage of apoptosis in untreated Mks was greater than that in CD34+ cells (Table III). NO-induced apoptosis became detectable within the first 24 hours of culture. Similar results were obtained when morphologic assessment of apoptosis was performed by staining cells with ethidium bromide and acridine orange (data not shown).

Role of endogenous NO on Mk proliferation. The induction of NO by TNF-α (10 ng/mL) and IFN-γ (100 U/mL) resulted in marked inhibition of Mk proliferation derived from CD34+ or MNCs that was partially reverted by NOS inhibitors (Fig 7 and 8). An analysis of NO generation in MNC supernatants showed that 72 hours after cytokine stimulation, there was a significant increase ($P < .05$, n = 3) in NO levels in comparison with non-stimulated cells and that such an increase was totally suppressed by NOS inhibitors. On the other hand, when CD34+ cultures were incubated with L-NAME, an increase in Mk generation occurred, whereas the addition of L-arg to CD34+ cells inhibited Mk formation. The inhibitory effect of L-arg could be reverted by L-NAME (Fig 8).

DISCUSSION

In this article we evaluated the role of NO on Mk formation induced by TPO. Our results demonstrated that in the MNC population, the presence of two NO donors with a non-related chemical structure and with different kinetics of NO release inhibited Mk growth in a concentration-dependent manner. We ruled out the possibility that the DETA/NO carrier molecule, nitrite or cyanide released from SNP, was responsible for this effect, because none of these compounds was able to decrease Mk formation.

Because NO also blocked Mk growth from a highly purified CD34+ cell population, its inhibitory action appeared unrelated to an effect on the accessory cells present in the MNC population but rather to a direct effect on hematopoietic progenitors. Moreover, the observation that inhibition of Mk growth was also achieved when differentiation was induced by IL-3 supported the hypothesis that the NO effect was directly
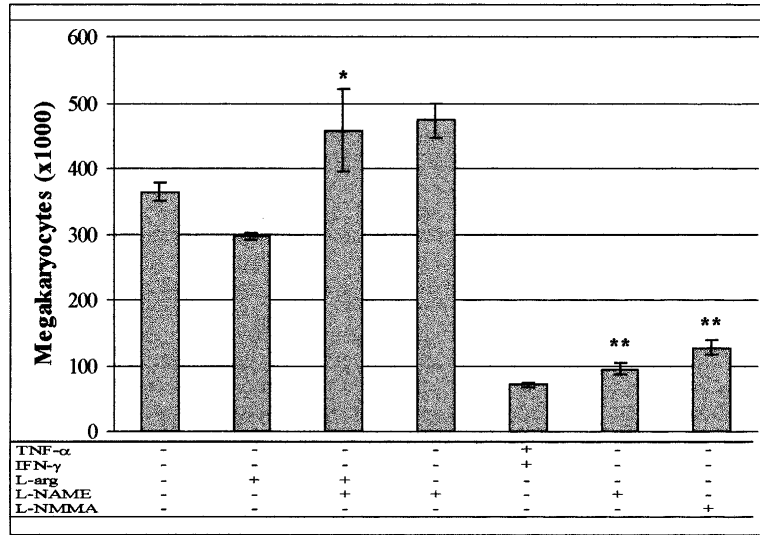
Fig 8. Effect of NO on Mks derived from CD34+ cells. CD34+ cells (5×10^4) were treated or untreated with L-arg or TNF-α and IFN-γ with or without L-NMMA (0.5 μmol/L) or L-NAME (0.5 μmol/L). Absolute number of Mks was determined at days 11 through 13 after TPO stimulation. Values represent the mean ± SEM of four independent experiments. * indicates statistically significant ($P < .05$) comparing L-arg vs L-arg and L-NAME. ** indicates statistically significant ($P < .05$) comparing cytokine vs cytokine and inhibitors. All treatments were statistically significantly different when compared with control sample.

exerted on progenitor cells. Data showing that CD34+ cells were more sensitive to the NO inhibitory effect than were MNCs suggest that accessory cells may well release cytokines that protect progenitors from the cytotoxic NO effect. Using semisolid MNCs and CD34+ cell cultures, Shami et al[11] demonstrated differential effects of SNP and S-nitroso-acetyl penicillamine on erythroid and myeloid colonies. Although we used the same concentration range, we failed to observe a differential effect regarding NO donor concentration or cell type used. The differences could be explained by the fact that Shami et al used not only a different culture technique but also a cocktail of cytokines that excluded TPO.

The activation of guanylate cyclase and an increase in cGMP are the mechanisms of NO action in the vascular system.[1] However, it has also been demonstrated that such an increase is the pathway by which NO induces an increase in the proliferation of smooth muscle cells as well as the migration and proliferation of endothelial cells.[27,28] Our finding that CD34+ cell treatment with 8-Br-cGMP had no effect on Mk production suggests that activation of guanylate cyclase may not be involved in the signaling transduction pathway related to NO-mediated Mk growth inhibition. However, we had found that NO was capable of inducing apoptosis in both CD34+ cells and Mks. Interestingly, after similar exposure to NO, Mks appeared to be more susceptible to NO-induced cell death than did progenitor cells.

These results are in agreement with the observation by Zauli et al[29] that the terminal phase of Mk life span is characterized by the onset of apoptosis. Furthermore, while this work was being undertaken, Battinelli et al[30] established that exogenous and endogenous NO sources were capable of inducing the apoptosis of megakaryocytic cell lines. However, in contrast to our findings, they also contended that TPO decreases the apoptotic response to NO. Discrepancies in the results could be explained by the use in our studies of primary culture cells instead of immortal cell lines to study the regulatory role of NO and TPO. Moreover, Zauli et al[29] demonstrated that the physiologic apoptosis of primary Mks is modulated to a certain extent by TPO and only during early culture times.

The cytotoxic effect of DETA/NO was exerted not only on undifferentiated CD34+ cells but also on more mature Mks. We observed that the presence of NO after culture proliferation had ceased and resulted in progressive recovery of Mks, although cell viability was abnormal 24 hours after the addition of DETA/NO. One possible explanation for the differences found in both cell recovery and viability depending on the culture day of NO addition seems related to the different times that CD34+ cells or CD41+ cells were exposed to NO and other toxic metabolites generated during cell death.

A marked decrease in Mk production was also observed when endogenous NO was induced by pro-inflammatory substances in either MNC or CD34+ cell

cultures. The observation that NOS inhibitors partially reversed the inhibition of Mk growth while increased NO levels returned to normal values indicates that decreased Mk growth mediated by endogenous NO is partially associated with the cytotoxic TNF-α and IFN-γ effect. However, the role of NO in the inhibition of cellular growth mediated by pro-inflammatory substances is somewhat controversial. Although Maciejewski et al[10] demonstrated the presence of iNOS in both MNCs and CD34+ cells of human BM, its enhancement by IFN-γ and TNF-α, and its partial reversal of the colony formation inhibition by NOS inhibitors, Punjabi et al[26] have shown that the combination of GM-CSF and LPS or IFN-γ markedly suppressed BM cell proliferation, which was completely reversed by L-NMMA.

We have also observed that the number of Mks derived from CD34+ cells was decreased by treatment with L-arg and was reversed by L-NMMA. Moreover, pretreatment of CD34+ cells with the NOS inhibitor enhanced Mk production. As far as we know, constitutive NOS in CD34+ cells has not yet been identified. Although these results suggest that progenitor cells produce NO in a constitutive manner, experiments such as RT-PCR and immunocytochemical staining are required to confirm the presence of a constitutive NOS in CD34+ cells.

In summary, our results present strong evidence that NO regulates Mk growth induced by TPO through an irreversible cytotoxic effect on progenitors and committed and mature Mks. Together with those previously reported, our findings demonstrate that NO affects the growth and differentiation of hematopoietic cells, including Mks.

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