Involvement of Nitric Oxide in Target-Cell Lysis and DNA Fragmentation Induced by Murine Natural Killer Cells

By János G. Filep, Chantal Baron, Silvana Lachance, Claude Perreault, and John S.D. Chan

Although it has been recognized for some time that target cells destroyed by natural killer (NK) cells die largely by apoptosis, the underlying mechanisms are not fully understood. The aim of the present study was to examine the role of nitric oxide (NO) in mediating murine NK-cell-induced killing of YAC-1 lymphoma cells. NK cells induced extensive release of 3H-DNA and 51Cr from YAC-1 cells. The target killing ability of NK cells was associated with an increased production of NO as measured by concentrations of nitrite in the culture medium. That YAC-1 killing resulted, in part, from the production of NO was confirmed by the significant protection of cell lysis in L-arginine-depleted medium and by approximately 30% attenuation of cell lysis and DNA fragmentation by an inhibitor of NO synthase, N°-nitro-L-arginine methyl ester (L-NAME) in a culture medium containing 1 mmol/L L-arginine. Fluorescence microscopic examination of YAC-1 cells showed the presence of changes in nuclear morphology characteristic for apoptosis. The percentage of apoptotic cells was markedly decreased by L-NAME. Further evidence for apoptosis is provided by the specific pattern of internucleosomal DNA fragmentation both in the absence and presence of L-NAME. During target-cell killing, an increased oxidation of intracellularly trapped dichlorofluorescein was observed in cells labeled with an antimouse NK-cell monoclonal antibody, as measured by flow cytometry. These increases were effectively prevented by L-NAME, but not W-13, an inhibitor of calmodulin. The ability of NO to induce cell lysis and DNA fragmentation in YAC-1 cells was further demonstrated by exposing tumor cells to chemically generated NO. Taken together, these observations suggest a role for NO as one of the mediators of NK-cell-mediated DNA fragmentation and cell lysis.

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NATURAL KILLER (NK) CELLS are large, nonadherent granular lymphocytes, capable of destroying certain susceptible tumor cells without prior sensitization and without restriction by major histocompatibility antigens. Their cytotoxic action is not dependent on expression of the α, β, and γ T-cell receptor genes or Rag-1/-2-dependent gene rearrangement.1,2 Tumor-cell destruction requires cell-to-cell contact and is thought to be mediated, in part, by soluble cytotoxic factors, including the membrane pore-forming protein perforin3 and serine proteases termed granzymes and proteoglycans. While isolated lytic granules and purified perforin could cause cell membrane damage under certain conditions, the resulting cell death does not involve DNA fragmentation,4 a hallmark of NK attack. Recent studies have implicated granzymes, in particular granzyme B, as inducers of the rapid DNA fragmentation and apoptosis by NK cells.5,6

Nitric oxide (NO) is a multifunctional molecule that is found in a variety of mammalian cells, NO produced by endothelial cells or neurons via the constitutive NO synthase serves as a physiologic regulator of vascular tone or as a neurotransmitter, respectively. On the other hand, NO generated by both immune and nonimmune cells via the inducible NO synthase is cytotoxic. For instance, in nonspecific host defense, macrophage-mediated killing or inhibition of proliferation of microorganisms and tumor cells involves NO both in vitro4,14 and in vivo.15,16 NO can cause DNA strand breaks and mutations17,18 and is capable of inducing internucleosomal DNA fragmentation in murine peritoneal macrophages19 and transformed fibroblasts.20 Recent observations suggest that NO may be a mediator of graft-versus-host disease,21,22 in which NK cells have also been implicated in the pathogenesis of the disease.23 Furthermore, generation of lymphokine-activated killer-cell activity has been reported to depend on NO production in rats and mice.24,25 In the present study, we measured the production of NO during murine NK-cell-mediated killing of lymphoma cells and investigated the role of NO in mediating cell lysis and DNA fragmentation.

MATERIALS AND METHODS

Animals. Male C57BL/6J (H-2b haplotype) mice (6 to 10 weeks old) purchased from The Jackson Laboratory (Bar Harbor, ME) were used as spleen donors.

Effector cells. Mice were killed by cervical dislocation. Spleens from untreated animals or mice injected intraperitoneally 18 hours earlier with 100 µg polyinosinic-polycytidylic acid (poly I:poly C; Sigma, St Louis, MO), a potent activator of NK cells,26,27 were removed under aseptic conditions and spleen cells were harvested. Following lysis of contaminating red blood cells, the cells were washed twice in RPMI-1640 medium and incubated on plastic culture dishes for 1 hour at 37°C in 5% CO2 in air. Nonadherent cells were then harvested and resuspended in culture medium consisting of RPMI-1640 reconstituted with or without L-arginine (1 mmol/L) (Select Amine Kit; GIBCO, Grand Island, NY), 10% fetal bovine serum (HyClone Laboratories, Logan, UT), 2 mmol/L glutamine, 10 mmol/L HEPES, 100 U/mL pencillin, and 100 µg/mL streptomycin.

The t-arginine concentration in unsupplemented culture medium was approximately 10 µmol/L due to the L-arginine provided by the fetal bovine serum. The resulting cells (viability >99% by trypsin blue exclusion test) were called NK cells in the present study. Cell preparations contained 1% to 4% monocytes/macrophages (as assessed by neutral red staining) and less than 0.2% neutrophils (Wright staining). The percentage of NK cells was assessed by an
R-phycoerythrin-conjugated mouse antimouse NK-cell monoclonal antibody (clone, 2B4; isotype, immunoglobulin [Ig]G2a, Phar- 
mingen, San Diego, CA), which reacted with 3% to 4% and 4% to 6% of the resulting cells from untreated and poly I:C-treated 
mice, respectively. This antibody reacts with all NK-1.1+ cells and a subset of T cells that mediate non–MHC-restricted cytotoxicity, 
but not with B cells, neutrophils, or monocytes/macrophages.

Radiolabeling target cells. YAC-1, a murine lymphoma cell line 
(American Type Cell Culture, Rockville, MD) was grown as a sus- 
pension culture in complete RPMI-1640 medium supplemented with 
10% heat-inactivated fetal bovine serum, 2 mmol/L T-glutamine, 
100 U/mL penicillin, and 100 μg/mL streptomycin. YAC-1 cells 
(0.5 to 2 × 10^6 cells) were labeled in their cytoplasm with ^{32}Cr 
and/or in their DNA with ([H]dezoxuridine ([H]dUdR) by incubation 
with 10 μCi [H]dUdR (ICN Radiochemicals, Irvine, CA) for 2 hours 
at 37°C in 100 μL culture medium. During the last 60 minutes of 
incubation, 10 μCi of Na[^{51}Cr]0₄ (New England Nuclear, Boston, 
MA) was added. The cells were then washed three times in culture 
medium. The doubly labeled cells were used only if the incorporation 
level of [H]dUdR was greater than 1 cpm/cell and if the [H]dUdR to 
^{32}Cr radioactivity ratio was greater than 2.

Quantitation of DNA fragmentation and cytology. Target cells 
labeled with ^{32}Cr and/or [H]dUdR (2.5 × 10^6 in 100 μL) were seeded 
into 96-well microtiter plates that contained various number of NK 
cells in triplicate in 100 μL of medium. A portion of NK cells 
was preincubated with the NO synthase inhibitor, N^6-nitro-L-arginine 
methyl ester (L-NAME; final concentration, 1 mmol/L; Sigma) or 
its biologically inactive d-enantiomer, N^6-nitro-D-arginine methyl 
ester (D-NAME; Research Biochemical International, Natick, MA) 
for 10 minutes at 37°C. The plates were centrifuged at 50g for 5 
minutes to establish cell contact and were incubated at 37°C in 5% 
CO₂ in air for various times before harvest. At the end of the induc- 
tion period, the cells were centrifuged at 200g for 10 minutes and 
the cell-free supernatant carefully withdrawn and saved. For DNA 
fractionation assays, the cell pellet was lysed with 1 mL of 10 
mmol/L Tris (pH 7.5) that contained 1 mmol/L EDTA and 0.2% 
Triton X-100. The lysates were then centrifuged at 13,000g for 10 
minutes to separate fragmented from intact chromatin. The 
radioactivity present in the incubation medium, in the 13,000g supernatant, 
and in the 13,000g pellet were quantitated using a Wallac Wizard 
Automatic Gamma Counter (Turku, Finland). The system 
was programmed to correct for crosstalk and spillover between de- 
terminers and counting channels. The percent ^{32}Cr release was 
calculated using the following formula: % lysis = \frac{(cpm_{meas} - cpm_{null})}{cpm_{null}} × 100, where cpm_{meas} is the radioactivity present in the incubation 
medium and cpm_{null} is the total ^{32}Cr radioactivity present in the incubation 
medium, 13,000g supernatant, and 13,000g pellet. The percent 
fragmented DNA was calculated using the formula: % fragmented 
DNA = \frac{(cpm_{fragnented} + cpm_{null})}{cpm_{null}} × 100, where cpm_{fragnented} is the 
^{32}S radioactivity present in the culture medium plus the 13,000g supernatant (ie, nonsedimenting chromatin) and cpm_{null} is 
the % fragmented DNA and cpm_{null} is the total ^{32}S radioactivity present in the 13,000g pellet. Percent 
specific fragmented DNA and lysis were calculated by the following 
formula: % specific fragmented DNA or lysis = \frac{(E - S) + (100 - S)}{100}, where E is the experimental value for percent fragmented 
DNA or lysis and S is the spontaneous value for percent fragmented 
DNA or lysis.

DNA isolation and gel electrophoresis. Following 4 hours of 
incubation, nonsedimenting chromatin from 13,000g supernatants of 
hypotonically lysed cells was precipitated with 50% isopropanol and 
0.5 mol/L NaCl overnight at −20°C. The percentage of dead cells and nuclei 
was calculated using the formula: % specific fragmented DNA or lysis = \frac{(E - S) + (100 - S)}{100}, where E is the experimental value for percent fragmented 
DNA or lysis and S is the spontaneous value for percent fragmented 
DNA or lysis.

Flow cytometry of NK-cell fluorescence during target killing. 
NK-cell chemiluminescence was measured using the 2',7'-dichloro- 
fluorescein (DCFH) oxidation assay. The oxidation of nonfluorescent 
DCFH trapped inside the cells to a fluorescence derivative, 2',7'- 
dichlorofluorescein, can occur either by hydrogen peroxide or NO. The 
percentage of dead cells and nuclei with apoptotic nuclei was calculated as follows: % dead cells = (NVN + NVA) / total number of cells counted) × 100, and % apoptotic cells = (VA + NVA) / total number of cells counted) × 100.
Increased with increasing effector:target (E:T) ratio until E:T ratios appeared to exert similar degree of inhibition at these higher incubation (Fig 1), detected at E:T ratios higher than 12:1, and L-NAME appeared to inhibit nitrite production under the present experimental conditions. YAC-1 cells alone did not produce detectable amounts of nitrite under the present experimental conditions.

Specific 51Cr release and DNA fragmentation were attenuated by 24% to 36% and 28%, respectively. A statistically significant inhibitory action of L-NAME on 51Cr release and DNA fragmentation was detected at E:T ratios higher than 12:1, and L-NAME appeared to exert similar degree of inhibition at these higher E:T ratios.

The target-killing ability of NK cells was associated with an increased production of NO as measured by concentrations of nitrate in the culture supernatants after a 4-hour incubation at 37°C. With increasing numbers of NK cells added, there was a concomitant increase in specific 51Cr release (Fig 1). Specific DNA fragmentation increased with increasing effector:target (E:T) ratio until 100:1, whereas at the E:T ratio of 200:1, it began to decrease (Fig 1). In the presence of L-NAME, 51Cr release and DNA fragmentation were attenuated by 24% to 36% and 18% to 28%, respectively. A statistically significant inhibitory action of L-NAME on 51Cr release and DNA fragmentation was detected at E:T ratios higher than 12:1, and L-NAME appeared to exert similar degree of inhibition at these higher E:T ratios.

The reversibility of the inhibitory action of L-NAME on NK-cell-induced cytolysis and DNA fragmentation was observed in the presence of L-NAME (Fig 2). However, the inhibitory action of L-NAME became more pronounced with increased incubation time (Fig 2).

The reversibility of the inhibitory action of L-NAME on NK-cell killing was studied at an E:T ratio of 100:1. In the presence of L-NAME (1 mmol/L), specific 51Cr release decreased from 40.2% ± 3.8% to 26.4% ± 1.7% (n=5, P<.05). Addition of L-arginine (10 mmol/L) to the culture medium prevented the inhibitory action of L-NAME on NK-cell killing (38.4% ± 2.0% specific lysis, n=5, P>.1 untreated cells), whereas d-arginine (10 mmol/L) was without effect (22.6% ± 3.0% specific lysis, n=5, P>.1 L-NAME–treated cells). Unlike L-NAME, D-NAME (1 mmol/L) had no significant effect on NK-cell killing (40.4% ± 4.3% specific cell lysis).

To provide further evidence for the involvement of NO in NK-cell killing, we compared the effect of L-NAME on cell lysis induced by NK cells from untreated animals and from mice injected with poly I:poly C. Poly I:poly C treatment markedly enhanced the cytotoxic potential of NK cells in culture medium that contained 1 mmol/L L-arginine (Fig 3A and B). While L-NAME significantly inhibited target lysis by NK cells from mice injected with poly I:poly C, it failed to affect the cytolytic activity of NK cells from untreated mice (Fig 3A). Further demonstrating that NO contributed to NK killing, the cytotoxic activity of poly I:poly C–activated NK cells was decreased in L-arginine–deficient culture medium (31.9% ± 2.6% specific target cell lysis, n
The results obtained in the DNA fragmentation and nuclear morphology assays were confirmed by agarose gel electrophoresis of DNA isolated from the 13,000g supernatant of lysed YAC-1 cells after a 4-hour incubation with various numbers of NK cells. Oligonucleosome-sized fragments characteristic for apoptosis were found in samples prepared from YAC-1 cells cultured in the absence and presence of L-NAME (Fig 4A).

Effects of chemically generated NO on YAC-1 cells. The direct killing ability of NO-generating agents, such as sodium nitroprusside, on YAC-1 cells was also examined. Exposure of YAC-1 cells to 6 mmol/L sodium nitroprusside for 4 hours resulted in 28.4% ± 3.9% specific cell lysis and 26.2% ± 3.7% DNA fragmentation (n = 4). Furthermore, DNA isolated from sodium nitroprusside-treated cells showed a similar pattern of internucleosomal DNA fragmentation to that observed during NK-cell killing (Fig 4B).

NK-cell fluorescence during target killing. NK-1.1+ cells labeled with R-phycocerythrin-conjugated antimouse NK-cell monoclonal antibody were gated by a flow cytometer, and mean channel fluorescence for oxidized DCFH was compared in the absence or presence of 1 mmol/L L-NAME or 100 μmol/L W-13. Mean channel fluorescence with a nearly equal degree of apoptotic morphology. The percentage of both dead cells and apoptotic cells was significantly decreased in the presence of L-NAME at all E:T ratios tested (Table 1).

Fig 2. Time course of killing YAC-1 cells with NK effector cells in the absence and presence of L-NAME. Various numbers of nonadherent spleen cells from C57 BL/6J mice injected with poly I:poly C (see Methods) were cultured with 1 mmol/L L-NAME (closed symbols) or its vehicle (open symbols) for 10 minutes and then 5 × 10⁵ YAC-1 cells labeled with 32PUDR and 51Cr were added to each tube. Specific DNA fragmentation and 51Cr release were determined at indicated times after incubation at 37°C. Percent spontaneous DNA fragmentation and cell lysis were 3% to 5% and 7% to 11%, respectively. The results are means ± SEM from 3 experiments performed in duplicate.

= 4, compared with 40.2% ± 3.2% target lysis, n = 5, in the presence of 1 mmol/L L-arginine; P < .05) (Fig 3C). Accordingly, after a 4-hour incubation, nitrite concentrations in the L-arginine-deficient medium supernatant were markedly lower than in medium that contained 1 mmol/L L-arginine (1.6 ± 0.5 μmol/L, n = 3, v 18.3 ± 2.8 μmol/L, n = 4; P < .05). Under these conditions, L-NAME did not modify significantly the specific cell lysis (Fig 3C).

Morphologic and molecular evidence for NO-mediated apoptosis. To ascertain that DNA fragmentation observed during NK-cell killing was apoptotic in nature, a morphologic assay and gel electrophoresis were performed. In the morphologic assay, the cells are stained with a mixture of acridine orange and ethidium bromide, and the percentage of viable and apoptotic cells quantified by recording the distribution of nuclear chromatin. As shown in Table 1, NK-cell-mediated killing of YAC-1 cells was always associated
The percentage of dead cells and cells with apoptotic nuclei were determined as described in the Materials and Methods, counting a minimum of 200 cells for each sample. The results are means ± SEM from 4 experiments.

Table 1. Effect of L-NAME on Tumor-Cell Viability and Nuclear Morphology During NK Cell-Mediated Cytolysis

<table>
<thead>
<tr>
<th>NK:YAC-1 Cell Ratio</th>
<th>% Dead Cells</th>
<th>% Apoptotic Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>-L-NAME</td>
<td>+L-NAME</td>
</tr>
<tr>
<td>200:1</td>
<td>52.8 ± 0.2</td>
<td>42.2 ± 4.6</td>
</tr>
<tr>
<td>100:1</td>
<td>49.7 ± 0.8</td>
<td>36.7 ± 4.4</td>
</tr>
<tr>
<td>50:1</td>
<td>36.9 ± 3.3</td>
<td>28.7 ± 2.9</td>
</tr>
<tr>
<td>25:1</td>
<td>25.6 ± 2.1</td>
<td>19.9 ± 1.6</td>
</tr>
<tr>
<td>YAC-1 only</td>
<td>4.4 ± 1.6</td>
<td>4.2 ± 1.2</td>
</tr>
</tbody>
</table>

A total of 5 × 10^3 YAC-1 cells were cultured with or without nonadherent spleen cells from C57 BL/6J mice injected with poly I:poly C at NK:YAC-1 cell ratios indicated in the absence and presence of 1 mmol/L L-NAME. After a 4-hour incubation at 37°C, the cells were pelleted by centrifugation, resuspended, and stained in 20 μL culture medium that contained 2 μL of a mixture of ethidium bromide and acridine orange. The percentage of dead cells and cells with apoptotic nuclei were determined as described in the Materials and Methods, counting a minimum of 200 cells for each sample. The results are means ± SEM from 4 experiments.

Abbreviation: NS, not significant.
L-NAME was greater with increasing E:T ratios. A possible explanation of this observation might be that a threshold concentration of NO is required to induce killing, and that the relative importance of different pathways, e.g., perforin, granzymes, and NO, which mediate target killing, varies at different E:T ratios. Further supporting that the production of NO was involved in lysis of YAC-1 cells, our studies showed that the cytotoxic potential of NK cells was decreased in L-arginine-deficient medium as compared with target-cell lysis observed in medium that contained 1 mmol/L L-arginine, and L-NAME had no significant effect on cell lysis under these conditions. These findings are consistent with previous observations using rat NK cells. During preparation of the manuscript, Xiao et al. reported a 70% decrease in human NK-cell-mediated cytotoxicity in an amino acid mixture-free RPMI 1640 medium as compared with that observed in a complete medium. Furthermore, NK-cell activity was completely restored by adding L-arginine to the medium. The differences in the degree of inhibition of NK lytic function by L-arginine depletion observed in this latter and the present study may be due to species differences and/or to differences in the experimental conditions (e.g., differences in the composition of culture medium).

The similar kinetics and similar percentage of DNA fragmentation as compared with *51Cr release both in the absence and presence of L-NAME make it impossible to conclude whether NO-dependent DNA fragmentation is an early or late event in NK-cell-mediated killing. However, NO may not function as a rapid inducer of apoptosis, for the inhibitory effect of L-NAME became more pronounced with time. Indeed, by using murine granzyme B** NK cells, Shresta et al. concluded that granzyme B plays a critical and nonredundant role in the rapid induction of DNA fragmentation. The defect in DNA fragmentation of granzyme B** NK cells is partially compensated by long incubation times, suggesting the existence of an intact "late" killing pathway(s). One of these mechanisms may involve NO. The present data also show that NO is not the sole inducer of late apoptosis as evidenced by the similar oligonucleosomal pattern of DNA fragmentation in the absence and presence of L-NAME. The involvement of NO in induction of apoptosis is further supported by fluorescence microscopic evaluation of YAC-1 cells, which showed significant decreases by L-NAME in the number of cells with apoptotic changes in nuclear morphology at all E:T ratios tested. The degree of L-NAME inhibition was similar to that observed in the DNA fragmentation assay.

NK-cell-mediated killing was associated with a concomitant dose-dependent increase in nitrite concentration in the culture medium, which indicates an enhanced NO production. Since our NK-cell preparations contained 1% to 4% monocytes/macrophages, we addressed the cellular source of NO by measuring intracellular fluorescence of oxidized DCFH in NK cells labeled with an antimouse NK-cell monoclonal antibody. DCFH fluorescence of nonadherent spleen cells (A) and antibody-labeled NK 1.1 cells (B) were analyzed by a flow cytometer. Values are mean ± SEM from 6 experiments performed in duplicate. The Kruskal-Wallis test indicated that variation among column medians is significantly greater (P < .01) than expected by chance. P values were obtained by Dunn's multiple comparison test.
peroxide\textsuperscript{33} or NO.\textsuperscript{31} The observations that L-NAME, but not W-13, a calmodulin inhibitor that inhibits superoxide formation and consequently hydrogen peroxide generation, effectively prevented the increase in dichlorofluorescein fluorescence in NK 1.1\textsuperscript{+} cells, would indicate that oxidation of DCFH can be attributed to NO rather than H\textsubscript{2}O\textsubscript{2}. These studies also suggested that an interaction of NK cells with YAC-1 cells may be necessary for the NK cells to produce NO. Murine P815 mastocytoma and L1210 leukemia cells have been reported to release soluble factors that activate murine macrophages for NO production for their own suicidal death.\textsuperscript{24} It remains to be investigated whether YAC-1 elaborate soluble factors that activate NK cells. By using the DCFH oxidation assay, we were unable to quantitate the amount of NO produced by NK cells during the 4-hour cytotoxicity assay, since the viability of DCFH-loaded cells decreased considerably after 1 hour. Therefore, we cannot exclude the possibility that contaminating cells in NK-cell preparations might have also contributed to NO production. If monocytes/macrophages had generated a significant portion of NO, a decrease in DCFH oxidation could be detected by either L-NAME or W-13, because activated monocyte/macrophages produce NO and superoxide, and consequently H\textsubscript{2}O\textsubscript{2}, simultaneously.\textsuperscript{25} Indeed, our results show that DCFH oxidation of activated monocytes/macrophages can be attenuated by both W-13 and L-NAME. However, H\textsubscript{2}O\textsubscript{2} generation appears to be negligible under the present experimental conditions, as W-13 did not affect significantly DCFH oxidation by effector cell preparations. These observations would argue against contribution of monocytes/macrophages to NO production in the circumstances of our experiments, and suggest that NK cells were indeed responsible for NO production. The lack of effect of W-13 on DCFH fluorescence in NK 1.1\textsuperscript{+} cells would lend further support to the notion that NK-cell killing does not depend on production of free oxygen radicals.\textsuperscript{36,37}

In contrast to NK cells from poly I:poly C–treated mice, the cytolytic action of NK cells from untreated mice was not inhibited by L-NAME. Under these conditions, nitrite concentrations in the culture medium were below the detection limit of the assay after a 4-hour incubation. These findings are consistent with previous observations on the lack of effect of another NO synthase inhibitor, \textit{L}-\textit{N}\textsuperscript{-}\textit{N}-monomethyl arginine, on the cytotoxic activity of NK cells prepared from untreated mice.\textsuperscript{21} However, NK-cell preparations from poly I:poly C–treated and control mice are not easily comparable, as poly I:poly C treatment markedly enhances NK-cell lytic activity due to induction of \textit{in vivo} \textit{gamma}-interferon production.\textsuperscript{26,37} Among its numerous actions, \textit{gamma}-interferon is thought to enhance NO production via the inducible NO synthase pathway.\textsuperscript{11} Thus, it is plausible to assume that enhancement of NK activity by poly I:poly C treatment can be attributed, in part, to induction of NO synthase. An enhanced production of NO by NK cells may also explain the enhanced in vitro cytotoxic activity of human NK cells preincubated with L-arginine,\textsuperscript{28,38} the substrate for NO synthases.

To confirm that YAC-1–cell apoptosis can be induced by NO, tumor cells were exposed to chemically generated NO. As a source of NO, we used sodium nitroprusside, which releases NO continuously by an internal transfer of one electron to NO\textsuperscript{39}. Results from these experiments showed that typical DNA fragmentation and cell lysis were detectable after a 4-hour culture of YAC-1 cells with sodium nitroprusside. At 6 mmol/L sodium nitroprusside, NO is generated at a rate of 0.2 to 0.3 \textit{mu}mol/L/min.\textsuperscript{39} Therefore, a maximum nitrite concentration of 48 to 72 \textit{mu}mol/L can be calculated after a 4-hour incubation. Since these concentrations are comparable to those detected in the culture medium at higher NK:YAC-1 ratios, it seems likely that the amount of NO produced during NK killing is sufficient to induce DNA fragmentation and cell lysis in YAC-1 cells.

In conclusion, the present results suggest that NO may play a role as one of the mediators of murine NK-cell–mediated DNA fragmentation and cell lysis.

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\textbf{REFERENCES}


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NITRIC OXIDE IN NK-MEDIATED TARGET KILLING


Involvement of nitric oxide in target-cell lysis and DNA fragmentation induced by murine natural killer cells

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