Decreased exhaled nitric oxide as a marker of postinsult immune paralysis

Habiba L. Attalah,1 Stéphanie Honoré,1,2 Saadia Eddahibi,1 Elisabeth Marcos,1 Claude-James Soussy,2 Serge Adnot,1,3 and Christophe Delclaux1,3

1Institut National de la Santé et de la Recherche Medicale U492-Université Paris XII, and 2Service de Bactériologie and 3Service de Physiologie, Hôpital Henri Mondor, AP-HP, 94 010 Créteil, France

Submitted 11 August 2003; accepted in final form 6 May 2004

ALTERATIONS IN IMMUNE DEFENSES associated with critical illness, although long described, have only recently been individualized by the term “immune paralysis” (25). Immune paralysis includes functional impairments of monocytes and macrophages and neutrophils (11, 30), the two major phagocytic cells contributing to innate immune defenses. This suggests that immune paralysis may be a major risk factor for nosocomial infections. In keeping with this possibility, we previously described an association between impaired intracellular killing of Staphylococcus aureus by neutrophils and subsequent nosocomial infection (30).

Nitric oxide (NO) regulates neutrophil migration and alveolar macrophage functions such as cytokine synthesis and bacterial killing (15, 36, 19, 34, 23), which are impaired in critically ill patients. One may hypothesize that lung NO production could be affected in these patients because pneumonia is the most common nosocomial infection. Along this line, L-arginine, which is a semimicellar amino acid, is the substrate for NO synthesis, and arginine blood concentration is often decreased in critically ill patients (3), a fact that could explain the beneficial effect of formulas with high arginine content in terms of reduction of infectious complications (16).

To this end, we used a rat model of peritonitis, in which we measured pulmonary NO production and evaluated potential effects of NO on inflammatory cell phenotype modifications, i.e., abnormal neutrophil migration and alveolar macrophage cytokine synthesis, which are components of immune paralysis.

MATERIALS AND METHODS

Animals

Adult male Sprague-Dawley rats (IFFA CREDO Laboratories, L’Arbresle, France) weighing 225–250 g were used in all experiments. The rats were housed in air-filtered, temperature-controlled units and were allowed free access to food and water. The animals were housed as recommended by the European Convention for the Protection of Experimental Animals (Decree no. 2001-131, February 6, 2001). Our local animal subcommittee approved the animal protocols.

Animal Model of Peritonitis

Sepsis was produced in the rats by cecal ligation and puncture (CLP), as described elsewhere (2). After halothane anesthesia, a 3-cm midline abdominal incision was made to expose the cecon, which was ligated with 3-0 silk (Ethicon) suture, just distal to the ileocecal valve to avoid intestinal obstruction. The cecon was punctured twice with a 19-gauge needle. The cecon was then replaced in the peritoneal cavity, and the incision was closed with a continuous 3-0 silk suture. The control (sham-operated) rats underwent the same procedures except that the cecon was not ligated nor punctured. All rats were given 1 ml of physiological saline subcutaneously for fluid resuscitation during the postoperative period. By using this low number of punctures, a mild localized sepsis was obtained as previously described (2), giving a low spontaneous mortality rate (~20%).

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design was chosen inasmuch as we previously showed that a postagressive inhibition of neutrophil migration occurs in this setting that further allows studying almost all rats.

At designated time points after CLP (1, 4, 24, 48, and 72 h), the rats were anesthetized by intraperitoneal injection of pentobarbital, and tracheotomy was performed for mechanical ventilation with a rodent ventilator (tidal volume of 7 ml/kg; respiratory rate of 60/min).

**Measurement of Exhaled NO Concentration**

Exhaled NO was measured with a chemiluminescence analyzer (EVA 4000, Sérès, Aix en Provence, France), with a lower limit of detection of 1 part per billion (ppb) and a NO sampling rate of 30 l/h. Calibration was performed with a zero gas and standard 100 and 800 ppb NO gas (AGA).

All rats were ventilated with NO-free air. Exhaled breath from each rat was collected in a 6-liter polyethylene bag that did not react with NO. The bag was placed at the expiratory outlet of the ventilator and was connected rapidly to the NO analyzer when filled. Each gas collection lasted ~15 min. Therefore, measurements reported for each time point represent the mean NO concentration in exhaled gas collected over the preceding 15 min. NO concentrations were expressed in ppb.

**Plasma Collection**

After exhaled gas collection, rats were killed by exsanguination. Blood was obtained, and plasma was separated and stored at −80°C.

**Wet-to-Dry Weight Ratio**

One lobe of the freshly harvested left lung was separated, weighed, dried at 40°C for 72 h, and weighed again. The wet-to-dry lung weight ratio was calculated by dividing the initial weight by the dry weight. The remaining lobe was frozen in liquid nitrogen and stored at −80°C until use.

**Bronchoalveolar Lavage**

Bronchoalveolar lavage (BAL) was performed as previously described (2). The left bronchus was clamped for BAL of the right lung by using the tracheotomy tube. Five 4-ml aliquots of 37°C, sterile, pyrogen-free, 0.9% saline were flushed through the tracheotomy tube. The five fractions were recovered and pooled. The total number of cells was counted using a standard hemocytometer. The BAL fluid was then centrifuged at 300 g for 7 min. The supernatant was collected and stored at −80°C for further analysis.

**Alveolar Macrophage Isolation**

After BAL centrifugation, the cell pellet (>97% macrophages) was suspended in DMEM (Life Technologies, Cergy-Pontoise, France) containing antibiotics (100 U/ml penicillin, 100 mg/ml streptomycin, and 100 mg/ml gentamicin). The cells, at a concentration of 1 × 10^6/ml, were incubated for 4 h at 37°C in 5% CO2 with and without stimulation by lipopolysaccharide (LPS, Escherichia coli endotoxin 055:B5 Sigma, 1/10262), and 100 mg/ml pyrene-free, 0.9% saline were recovered and pooled. The total number of cells was counted using a standard hemocytometer. The BAL fluid was then centrifuged at 300 g for 7 min. The supernatant was collected and stored at −80°C for further analysis.

**Measurement of NO End Products (Nitrite/Nitrate)**

NO is unstable in the presence of oxygen and is rapidly converted into NO2 and NO3 (NO end products: NOx) in liquid media. For measurements of NO production in biological samples (plasma, BAL fluid, and alveolar supernatants), both NO2 and NO3 were converted into NO by using the reducing agent vanadium (III) chloride in acidic solution [hydrochloric acid (HCl) 6N] at 90°C (Nitroflow NT system, Sérès, as described elsewhere (37)]. NO gas was carried into the NO analyzer (EVA 4000 analyzer) with a constant flow of N2 gas and was quantified with reference to sodium nitrite standards. Before plasma NOx measurement, the frozen plasma was thawed and mixed with methanol (1:1 vol/vol) to precipitate the proteins and then centrifuged at 12,000 g for 20 min at 4°C.

**Western Blotting of NO Synthase-2 and NO Synthase-3 Proteins**

Immunoblotting for NO synthase (NOS)-2 and NOS-3 proteins was performed as described elsewhere (26). The frozen tissues were homogenized on ice by using a Polytron homogenizer (Kinematica, Steinhorhalde, Switzerland) in 0.1 mM PBS (pH 7.4) containing protease inhibitors (1 μM leupeptin, 1 μM pepstatin A). The homogenates were centrifuged at 490 g for 15 min at 4°C. Protein concentrations of the supernatants were measured by the Bradford method (Pierce, Rockford, IL), and supernatants (150 μg of protein loaded per lane) were then examined by SDS-PAGE. The proteins were transferred to nitrocellulose membranes by electrobolting in a transblot Bio-Rad transfer apparatus (Bio-Rad Laboratories, Richmond, CA). The nitrocellulose membranes were soaked in electrobolting buffer (25 mM Tris-HCl, 193 mM glycine, 20% methanol, pH 8.0) for 15 min before being transferred. After protein transfer, which was carried out for 12 h at 4°C, the membrane was blocked with 5% Tween Tris-buffered saline (TBS-T, 0.15 M NaCl, 10 mM Tris-HCl, 0.05% Tween 20, 5% BSA, pH 8.0) for 1 h at room temperature. The membranes were incubated with primary antibody, the endothelial NOS-3 protein (Transduction Laboratories, Lexington, KY) or the inducible NOS-2 protein (Transduction Laboratories) 1:1,000 for 1 h. The membranes were washed three times in 1% TBS-T. Specific protein was detected by use of horseradish peroxidase (Amersham), and the blots were developed by the Amersham enhanced chemiluminescence detection system. The bands for NOS-2 and/or NOS-3 were visualized and quantified by use of densitometry (personal densitometer SI; Molecular Dynamics).

**Measurement of NOS Activity**

NOS activity was measured in the lung homogenates on the basis of transformation of L-[3H]arginine to L-[3H]citrulline as described elsewhere (26). The tissue (100 mg) was homogenized on ice with a homogenizer, in four volumes of buffer containing 50 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 0.1 mM EGTA, 0.1% 2-mercaptoethanol, 100 mg/ml leupeptin, 1 μM pepstatin A, and 1 mM PMSF (buffer A). The homogenate was centrifuged at 10,000 g for 30 min at 5°C. The supernatant fraction was used to evaluate NOS-2 activity (cytosolic activity). The pellet (membrane fraction) was resuspended in homogenization buffer containing the detergent CHAPS (20 mM), 1 M KCl, and glycerol (10% vol/vol) and was then allowed to stand on ice for 30 min before centrifugation at 100,000 g for 30 min at 5°C. Activity of NOS-3 in the resultant supernatant fraction (membrane fraction) was determined by measuring Ca2+-dependent conversion of L-[3H]-arginine to L-[3H]citrulline. Specific activity of L-[3H]arginine was 320 Ci/mM. The reaction mixture contained the supernatant (<10% in volume), 0.2 μCi L-[3H]arginine, 50 μM L-arginine, 100 μM NADPH, 25 μM tetrahydrobipterin, 2 μM FAD, and 2 μM flavin mononucleotide. This mixture was incubated for 45 min at 37°C under a calcium-dependent condition, with 0.6 mM Ca2+, corresponding mainly to NOS-3 activity. After the reaction was stopped by the addition of 0.5 M perchloric acid, L-[3H]citrulline and L-[3H]arginine were separated on a cationic ion-exchange column (Shim-pack ISC-05/S0504; Bio-Rad) in a HPLC system (Waters Associates, Milford, MA). The citrulline fraction was collected by a fraction collector (model 201; Gilson, Villiers-le-Bel, France). The citrulline fraction was collected by a fraction collector (model 201; Gilson, Villiers-le-Bel, France). The citrulline fraction was collected by a fraction collector (model 201; Gilson, Villiers-le-Bel, France).
remaining counts were then divided by the specific activity (320 mCi/mM). Next, the specific activity of NOS was expressed as picomoles of citrulline formation per milligram protein per 45 min.

**Arginase Inhibition Assay**

In macrophages, NOs and arginases compete for the same substrate, l-arginine, to produce NO and urea, respectively (6). Because l-arginine availability is a major determinant for NO synthesis in activated macrophages, we hypothesized that arginase might reduce NO production. To test this hypothesis, we investigated the effect of the specific arginase inhibitor N-hydroxy-nor-l-arginine, diacetate salt [NOHA (33)] (Calbiochem) on NO production. Isolated alveolar macrophages from both CLP rats and healthy control rats were suspended in presence of endotoxin stimulation (LPS, 1.0 μg/ml) for 4 h in the presence or absence of 10 or 100 μM NOHA. NO concentrations in cell supernatants were measured as described previously.

**Macrophage Inflammatory Protein-2 Concentration Measurement in Isolated Alveolar Macrophage Supernatant**

Macrophage inflammatory protein-2 (MIP-2) was assayed by use of a specific rat ELISA kit as recommended by the manufacturer (BioSource). The detection limit was 1 pg/ml.

**Alveolar Instillation of Endotoxin**

As a functional parameter characterizing immune paralysis, neutrophil influx into the lungs was assessed by endotoxin instillation, as previously described (20). Briefly, the rats were anesthetized by halothane administration, the trachea was exposed, and 0.5 ml/kg body wt of LPS (200 μg/kg) dissolved in sterile physiological saline was instilled via a 25-gauge needle. The skin incision was closed with surgical staples, and the rats were allowed to awaken. Four hours later, the rats were anesthetized by intraperitoneal pentobarbital administration to allow biological sample collection, as described previously.

**Treatment with the NOS Inhibitor Aminoguanidine**

Healthy rats were injected subcutaneously with aminoguanidine (30 mg/kg), and 4 h later exhaled NO was measured to verify NO inhibition. In a separate set of experiments, 4 h after aminoguanidine injection, the rats were instilled with endotoxin (see above) and then killed 4 h later for BAL to assess whether in vivo NO inhibition was associated with modifications in alveolar neutrophil influx.

**RESULTS**

**Decreased Exhaled NO After Peritonitis**

Experiments were designed to describe the time course of NO exhalation after mild peritonitis or a sham operation. The results are summarized in Table 1. An early decrease in exhaled NO was found after mild peritonitis that seemed related to a decrease in NO production, because NO-derived end products were not increased in BAL fluid or plasma. Furthermore, in addition to this decrease in NO production by the lungs, we found a decrease in NO production by isolated alveolar macrophages, which paralleled the decrease in exhaled NO (Table 1 and Fig. 1). By contrast, sham-operated animals exhibited a trend toward an early decrease in exhaled NO (4th hour), which was subsequently followed by a rapid increase in NO synthesis (24th hour).

| Time course of exhaled NO and NOx levels in BAL, alveolar macrophages, and plasma after CLP or sham surgery in rats, as compared with healthy rats |
|---------------------------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Healthy Rats & H4 & H24 & H1 & H4 & H24 & H48 & H72 (n = 6) |
| Total BAL fluid cell count, 10⁶ | 89 (74–100) & 121 (82–176) & 198 (144–252)† | 63 (45–76) & 160 (116–180) & 180 (106–181) & 130 (65–192) & 126 (90–156) |
| Wet-to-dry lung ratio | 3.9 (3.5–4.0) & ND & 4.1 (4.0–4.4) | 4.4 (3.6–4.5) & 4.0 (3.9–4.1) & 4.3 (4.1–4.4) & 4.4 (4.3–4.6)† & 4.4 (4.1–4.5)† |
| Exhaled NO, ppb | 4.0 (3.6–4.3) & 2.6 (2.3–3.5) & 9.1 (5.9–9.3)‡ | 2.7 (1.8–3.4) & 2.9 (2.2–3.7) & 1.5 (1.2–1.7)† & 1.2 (1.0–1.4)† & 2.0 (1.2–2.2)† |
| NOx, μM | BAL fluid | 0.8 (0.3–2.1) & 0.7 (0.4–1.1) & 1.4 (1.0–2.6) | 1.0 (0.6–1.2) & 1.4 (0.6–1.8) & 0.9 (0.7–1.6) & 0.5 (0.2–1.2) & 2.5 (2.4–2.7) |
| | Alveolar unstimulated | 2.3 (1.8–3.2) & 0.8 (0.4–1.2) & 8.0 (5.1–11.1)† | ND & 1.7 (1.2–2.5) & 0.6 (0.5–0.7) & 3.5 (3.0–4.9) * & 6.2 (3.2–11.9)† |
| | Macrophage LPS stimulated | 4.0 (3.5–7.1) & 0.8 (0.7–1.0) & 7.6 (5.2–10.1) | ND & 1.3 (1.1–2.1) & 1.0 (0.7–1.5)* & 4.9 (3.4–7.0) & 5.4 (3.6–6.7) |
| Plasma | 0.9 (0.4–2.5) & 4.4 (2.5–4.9) & 6.4 (4.8–7.5)† | 1.9 (0.6–3.2) & 0.4 (0.3–2.4) & 0.6 (0.4–0.7) & 0.6 (0.5–1.1) & 0.7 (0.5–0.6) |

Values are medians (25th–75th percentiles) for each group. NO, nitric oxide; NO, NO end products; H4–H24, 4- to 72-h time points after cecal ligation and puncture (CLP); BAL, bronchoalveolar lavage; ppb, parts per billion; LPS, lipopolysaccharide. *P < 0.05 compared with healthy group; †P < 0.01 compared with healthy group; ‡P < 0.001 compared with healthy group.
Mechanisms of Decreased Lung NO Synthesis

NOS assays on lung homogenates. The decrease in NO output was not related to downregulation of type 2 or 3 NOS proteins, as shown by the Western blot assays on lung homogenates. Expression of NOS-3 was not significantly increased after CLP, whereas expression of NOS-2 was significantly increased (Fig. 2). We then assessed NOS activities and found a decrease in NOS-2 activity in lung homogenates of CLP rats compared with healthy rats (Fig. 3).

Assays on isolated alveolar macrophages. To further explore the mechanism of decreased NO production by isolated alveolar macrophages, experiments using a specific arginase inhibitor (NOHA) were conducted (Table 2). Arginase inhibition caused a dose-dependent increase in NOx levels in alveolar macrophage supernatants of both healthy and CLP rats. Interestingly, arginase inhibition led to significantly greater enhancement of NOx production by macrophages from CLP rats than from healthy rats, suggesting enhanced NOS activity in the CLP rats.

Effects of Decreased Lung NO Production on Immune Function Regulation

In these experiments, we evaluated whether the decrease in NO production associated with mild CLP was involved in the inhibition of alveolar neutrophil migration that occurs after injury. After LPS instillation 24 or 72 h after CLP, the neutrophil influx into the alveolar spaces was less marked than in healthy rats. In vivo inhibition of NO synthesis by treatment of healthy rats with aminoguanidine led to a significant decrease in both exhaled NO [1.7 ppb (1.2–2.4), n = 3] and NOx in supernatants of isolated alveolar macrophages [unstimulated condition, 0.4 μM (0.2–0.7)] compared with healthy rats. Furthermore, aminoguanidine administration was associated with inhibition of neutrophil migration toward the alveolar spaces after CLP administration (Fig. 4).

To further explain the role of NO in macrophage functions, we assessed MIP-2 synthesis by isolated alveolar macrophages. MIP-2 is a chemokine involved in recruiting neutrophils to the alveolar spaces (Fig. 5). An increased MIP-2 secretion by isolated alveolar macrophages was evidenced in both the sham-operated and the CLP rats, despite the absence of alveolar neutrophil influx (data not shown). In vivo (aminoguanidine) inhibition of NO synthesis in healthy rats was followed by increased MIP-2 production into supernatants of isolated alveolar macrophages.

Effect of L-Arginine Supplementation

Administration of L-arginine was associated with an increase in exhaled NO compared with D-arginine administration [4.8–2.4], n = 3] on September 7, 2017 http://jap.physiology.org/ Downloaded from
isolated alveolar macrophages from healthy rats;† ed by L-arginine administration. A sim-
recruitment was modi-
instillation to assess whether the kinetic of alveolar neutrophil
1,089 (947–65) 590 (523–630) fl
Peritonitis, % 37 (24–661)*
P(25th–75th percentiles) for each condition. NOHA, N-hydroxy-nor-l-arginine, diacetate salt, an arginase inhibitor. Results for all conditions are expressed as the percentage of basal (without NOHA) NO end product production by alveolar macrophages from healthy rats, n = 3 exper-
iments per condition. *P < 0.05 compared with NO end-product production by isolated alveolar macrophages from healthy rats; †P < 0.05 compared with the basal condition.

DISCUSSION

Increased levels of exhaled NO have been found during pulmonary infections, as well as during intravenous endotoxin administration (1, 14). The contribution of alveolar macrophages to pulmonary NO production after endotoxin injection has been demonstrated by Fujii and colleagues (14). Unexpectedly, in our model of mild peritonitis, we found no increase in exhaled NO, but on the contrary, exhaled NO concentration was decreased after peritonitis. In keeping with our finding, a significant decrease in exhaled NO has been found in patients after cardiopulmonary bypass (18). Interestingly, L-arginine administration in this latter setting has been found to decrease the occurrence of infectious complications (16). The beneficial effects of immunonutrition using formulas with high content of L-arginine may be attributed to decreased blood concentration of L-arginine, which is a semisessential amino acid (3). We

Table 2. Experiments on isolated alveolar macrophages

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Basal</th>
<th>10 μM NOHA</th>
<th>100 μM NOHA</th>
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<tbody>
<tr>
<td>Healthy, %</td>
<td>100</td>
<td>330 (296–364)†</td>
<td>630 (597–671)†</td>
</tr>
<tr>
<td>Peritonitis, %</td>
<td>37 (24–65)</td>
<td>590 (523–661)†</td>
<td>1,089 (947–1,115)*†</td>
</tr>
</tbody>
</table>

Values are median (25th–75th percentiles) for each condition. NOHA, N-hydroxy-nor-l-arginine, diacetate salt, an arginase inhibitor. Results for all conditions are expressed as the percentage of basal (without NOHA) NO end product production by alveolar macrophages from healthy rats, n = 3 exper-
iments per condition. *P < 0.05 compared with NO end-product production by isolated alveolar macrophages from healthy rats; †P < 0.05 compared with the basal condition.
NO further suggests that NO production was decreased in our model.

There is controversy in the literature over whether NO released during the inflammatory process favors or inhibits neutrophil migration. However, exposure of neutrophils to exogenous NO induces a rapid and persistent morphological hyperpolarization followed by migrational arrest due to reduction of the F-actin content (22). Moreover, NO supplied exogenously into reactions containing activated endothelium down-regulates proinflammatory activity, such as the secretion of chemokines, and functional activity, such as transendothelial migration of neutrophils (13). These in vitro experiments may explain that the inhibition of neutrophil migration by NO is due to inhibition of a neutrophil-endothelium adhesion mechanism. In vivo experiments further support the role of NO in the inhibition of neutrophil migration at the time of severe sepsis (4) or subsequent to sepsis (for instance after intravenous endotoxin injection; Ref. 32). These effects of NO have previously been demonstrated to be mediated via inducible NOS-2 activity (29). Eventually, inhaled NO has also been demonstrated to reduce neutrophil emigration in some studies (15) (however, we found an opposite response in a bacterial pneumonia model; Ref. 19). Consequently, we were surprised to find an inhibition of neutrophil migration toward alveolar spaces after a mild peritonitis that was not associated with increased NO synthesis (at least in the pulmonary compartment from the first hour of injury). Furthermore, we have previously shown using the same animal model an increase in blood proinflammatory cytokines (2), which have been shown to inhibit subsequent neutrophil emigration via NO synthesis (31). On the other hand, in a model of trinitrobenzene sulfonic acid-induced colitis in rats, nitro-l-arginine methyl ester reduced neutrophil influx (17). Therefore, reduced NO synthesis has also been associated with inability for neutrophil to migrate.

A limitation of our study is the fact that sham-operated rats were not studied at all time points. These experiments showed a transient decrease in exhaled NO (4th hour) after sham surgery followed by an unexpected rebound of NO synthesis (24th hour). We did not subsequently performed experiments in sham rats inasmuch as statistical calculations demonstrated that we were unable to demonstrate significant differences between both surgeries using small number of animals (<30 per group). Consequently, from this study we cannot infer whether our results are related to surgical injury and/or septic injury. However, we previously showed that both surgeries were characterized by an increase in plasma IL-10 (2), an anti-inflammatory cytokine involved in immune paralysis (25).

To further explore the decrease in exhaled NO in our rats with mild peritonitis, we conducted experiments on isolated alveolar macrophages, because these cells also showed a decrease in NO synthesis, suggesting a link between cellular and organ impairment of NO production. Several lines of evidence led us to hypothesize that stress, via increased production of catecholamines and their cellular effects mediated by cAMP, leads to arginase upregulation and decreased arginine availability for NOS, the end result being impaired NO production (5, 8, 28). We chose to explore this hypothesis in isolated alveolar macrophages because arginase regulation in these cells has been extensively described (7, 24, 33). In macrophages, l-arginine can be used by NOSs and arginases to form NO and urea, respectively. A similar balance between arginases and NOS exists in endothelial cells (21, 38). Thus activation of arginases may be an effective mechanism for regulating NO production through substrate competition. Support for this possibility was provided by our experiments showing an increase in NOx production in the presence of a specific arginase inhibitor. The results of these ex vivo experiments are in agreement with in vitro evidence that there is impairment in NO production by macrophages or endothelial cells when both NOS-2 and arginase are induced by endotoxin (7, 21).

Although the reason for the decrease in lung NO production remains speculative, besides a decrease in substrate availability either relative (competition) or absolute (deficiency), several proteins have been shown to block the dimerization and activity of NOS isoforms (6). Furthermore, NOS-2 enzyme activity has been shown to be dependent on molecular oxygen levels (12); conceivably, a decrease in partial pressure of oxygen due to lung edema could also have reduced NO synthesis. The decrease in neutrophil emigration was not clearly related to defective substrate availability because l-arginine administration failed to improve neutrophil migration despite restoring exhaled NO concentration.

Inhibition of NOS-2 activity by treatment of healthy rats with amino guanidine, which reduced both exhaled NO and NOx production by alveolar macrophages, reproduced the impairment of neutrophil migration to alveolar spaces. The impairment in neutrophil migration was not related to a decrease in MIP-2 secretion by alveolar macrophages, because MIP-2 secretion was stimulated after both CLP and amino guanidine administration. This last finding may be ascribable to decreased NO production by alveolar macrophages because NO is known to inhibit nuclear factor-kB (via nitrosylation), a transcription factor responsible for proinflammatory cytokine upregulation (34). Given the lack of selectivity of the high dose of amino guanidine administered, it is also conceivable that the inhibitor may have inhibited endothelial NOS-3 remote from the inflammatory locus, resulting in vasoconstriction, reduced blood flow delivery, and reduced neutrophil emigration toward alveolar spaces.

We previously showed that peritonitis was characterized by an increase in plasma IL-10 (2), a major anti-inflammatory cytokine involved in immune paralysis (25) and macrophage arginase upregulation (24). Interestingly, Tsuei and colleagues (35) have demonstrated an increased arginase expression in blood monocytes that may have contributed to postsurgical immune dysfunction, which may have been regulated via plasma IL-10 secretion. These results suggest that plasma IL-10 and exhaled NO may be markers for immune paralysis. NO exhalation measurement has the advantage of being non-invasive. Clinical studies are ongoing in our institution to determine whether the decrease in exhaled NO is related to the alveolar or to the bronchial compartment, both of which contribute to exhaled NO (10), and whether exhaled NO may be a prognosis factor in critically ill patients.

In summary, mild peritonitis (local infection) is associated with an early decrease in exhaled NO that is related to decreased lung production. This impaired NO production is associated with impaired neutrophil migration into the alveolar spaces that may contribute to immune paralysis in critically ill patients. Thus exhaled NO may represent a noninvasive marker for immune paralysis.
EXHALED NO AFTER PERITONITIS

ACKNOWLEDGMENTS

The authors thank Dr. Antoinette Wolfe for expert manuscript review (and English corrections).

GRANTS

This study has been supported by a grant from “Legs Poix.”

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