Mitochondrial Perturbations and Oxidant Stress in Lymphocytes From Patients Undergoing Surgery and General Anesthesia

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Background: Previous studies have shown that a profound suppression of immune function transiently occurs in patients who undergo surgery under general anesthesia. The decline in the absolute counts of peripheral blood lymphocytes constitutes a major factor accounting for this immune defect, and recent evidence indicates that apoptosis plays a crucial role in determining postsurgical lymphocytopenia.

Hypothesis: An altered oxidation-reduction status of mitochondria may contribute through apoptosis to the loss of lymphocytes following surgical trauma and general anesthesia.

Design: We studied 16 patients with American Society of Anesthesiologists' physical status I or II who underwent elective surgery under general anesthesia. The data were collected prospectively.

Setting: University hospital.

Main Outcome Measures: Samples of peripheral blood were drawn on the day before surgery and at 24 and 96 hours after the operation. Following lymphocyte isolation, the mitochondrial transmembrane potential was assessed by flow cytometry using 3,3'-dihexylocarbocyanine iodide, and stains with hydroethidine and 2'-7'-dichlorofluorescein diacetate were used to determine the generation of reactive oxygen species. The labeling of lymphocytes with monobromobimane was used to assess the presence of reduced glutathione.

Results: At 24 hours after surgery, we detected a significantly elevated frequency of peripheral blood lymphocytes (P=.002), which incorporated low levels of 3,3'-dihexylocarbocyanine iodide, compared with the preoperative period. At this same time point, the frequency of lymphocytes with the hydroethidine- and 2'-7'-dichlorofluorescein diacetate–positive phenotype was elevated compared with baseline levels. Conversely, at 24 hours after surgery, the frequency of cells that stained positive for glutathione was strongly decreased compared with preoperative values. Overall measurements returned to the baseline levels at 96 hours after surgery.

Conclusion: The strict association we observed between the overproduction of reactive oxygen species and the disruption of the mitochondrial transmembrane potential supports the view that alterations in mitochondrial energy metabolism, paralleled by the presence of a pro-oxidant oxidation-reduction status, could be involved in the accelerated apoptotic loss of lymphocytes following surgical trauma and general anesthesia.

Arch Surg. 2001;136:1190-1196

A profound but transient decrease of circulating lymphocytes occurs in the early postoperative period, and this event appears to play a crucial role in the immune suppression that is commonly recognized after surgical operations.1-2 This transient immune deficiency may have a major clinical impact on the outcome of surgical patients as the risk of complications, in particular local infection and sepsis, has been directly linked to this phenomenon.3

Recent investigations4-5 have suggested that the unregulated activation of the process of apoptosis can determine the occurrence of lymphocytopenia and the severity of immune suppression in patients undergoing surgery and general anesthesia. However, the mechanisms that ultimately trigger lymphocyte apoptosis after operation are still unclear. In a recent study,6 it was found that the increased rate of lymphocyte apoptosis in the early postoperative period was paralleled by a severe imbalance in the expression of death and survival factors, with an enhanced expression of proapoptotic pathways, such as the Fas-Fas ligand system, and a downregulation of antiapoptotic factors, such as bcl-2.2 It is unknown whether additional mechanisms are involved in the accelerated apoptotic loss of lymphocytes caused by surgical trauma and general anesthesia.
PATIENTS AND METHODS

The study was approved by the Hospital Ethical Committee, and all participants provided written informed consent.

PATIENTS AND BLOOD SAMPLES

Sixteen patients with American Society of Anesthesiologists’ physical status I or II scheduled for elective surgery and general anesthesia were prospectively studied at the Department of Surgery, “La Sapienza” University, Rome, Italy. Patients were excluded if they were younger than 20 years or older than 75 years, had a malignant disease, or had a history of endocrine, hemato logic, or metabolic disorders. Exclusion criteria also included pregnancy, current infection; and use of immunosuppressive medication, including corticosteroids (within the past 3 months). A standardized model of general anesthesia was used for all patients. Premedication consisted of intramuscular diazepam, 10 mg, administered 30 minutes before the scheduled time of the operation. Anesthesia was induced with an intravenous bolus of propofol, 1 to 2 mg/kg, and fentanyl citrate, 2 µg/kg, and continued with a mixture of isoflurane in 40% to 60% oxygen in air. Intraoperative analgesia was sustained by additional doses of fentanyl administered as required. Muscle relaxation was obtained by means of pancuronium bromide.

Peripheral blood samples were drawn 1 day before surgery and at 24 and 96 hours after the operation (t1 and t2, respectively), and were immediately transferred to the laboratory for detection of ΔΨm, ROS generation, and GSH intracellular concentrations.

CONTROL SUBJECTS

Ten healthy volunteers selected from among the hospital and laboratory personnel were tested as reference controls. Blood samples were obtained from them on 2 occasions, separated by 24 hours, and processed in a similar manner as patients’ samples.

LYMPHOCYTE ISOLATION

Peripheral blood mononuclear cells were separated from heparinized peripheral blood using gradient centrifugation (Lymphoprep; Nycomed Pharma, Oslo, Norway), as described by the method of Boyum,28 washed twice with phosphate-buffered saline, and resuspended in Roswell Park Memorial Institute 1640 medium (Life Technologies Inc, Paisley, Scotland) supplemented with 10% heat-inactivated fetal calf serum (Life Technologies Inc); a combination of penicillin G sodium and streptomycin sulfate (Life Technologies Inc), 10 IU/mL; 10mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma-Aldrich Corp, St Louis, Mo); and 1mM glutamine (Life Technologies Inc) (complete medium). In addition, for the analysis of mitochondrial functions, aliquots of cells were isolated and maintained in complete culture medium at 4°C until labeling.

ANALYSIS OF MITOCHONDRIAL FUNCTIONS

For the simultaneous determination of surface markers and ΔΨm, cells were first stained with phycoerythrin-labeled anti-CD4+ or anti-CD8+ antibodies (Becton Dickinson, Immunocytometry Systems, Becton Dickinson and Co, San Jose, Calif) (30 minutes on ice). Cells were washed for 5 minutes at 600g and 4°C in an ice-cold staining buffer (phosphate-buffered saline, pH 7.2) supplemented with 2% bovine serum albumin (Sigma-Aldrich Corp) followed by exposure for 15 minutes at 37°C to 3,3′-dihexyloxacarbocyanine iodide (DiOC6,3) 40 nmol/L (Molecular Probes, Eugene, Ore).29 For the simultaneous assessment of surface markers and mitochondrial ROS generation, such as superoxide and hydrogen peroxide, cells were first stained with phycoerythrin-labeled anti-CD4+ or anti-CD8+ antibodies and then exposed for 15 minutes at 37°C to hydroethidine (HE), 2 nmol/L (Molecular Probes), and for 1 hour at 37°C to 5mM 2′,7′-dichlorofluorescein diacetate (DCFH-DA) (Molecular Probes), respectively.30,31 In control experiments, cells were labeled after preincubation with the uncoupling agent carbonyl cyanide m-chlorophenyl cyanide 5-chloro-2′-deoxyuridine (Sigma-Aldrich Corp), 50 nmol/L, at 37°C for 30 minutes, or the ROS-generating agent menadione (Sigma-Aldrich Corp), 1 nmol/L, at 37°C for 1 hour. For DCFH-DA, a positive control (cells kept 2 minutes in 15mM hydrogen peroxide and washed 3 times) was inserted. For the detection of GSH, lymphocytes were stained with monobromobimane (MBB) (Molecular Probes).32 In the presence of GSH S-transferase, MBB combines nonenzymatically with GSH at low concentrations, resulting in GSH-specific fluorescence. Briefly, T lymphocytes were pelletized and resuspended in 1 mL of medium containing 40µM MBB for 10 minutes at room temperature in the dark. Cells were placed on ice before analysis, which was performed on a cytofluorometer (FACSCAN; Becton Dickinson and Co). Forward and side scatter were gated on the population of living normal-sized lymphoid cells. After suitable compensation, fluorescence was recorded at different wavelengths: fluorescein isothiocyanate conjugated, DiOC6 (3), and DCFH-DA at 525 nm (fluorescence 1), phosphatidylethanolamine at 575 nm (fluorescence 2), and HE at 600 nm (fluorescence 3).

STATISTICAL ANALYSIS

Data are presented as mean±SD. Changes over time of the variables studied were examined using 1-way analysis of variance for repeated measurements. P<.05 was considered significant. The statistical analysis was done using a computer program (Statgraphics Plus; Bitstream, Inc, Cambridge, Mass).

Oxidant stress and reactive oxygen species (ROS) have been linked to the process of apoptosis, in particular through mediating the expression and/or activity of proapoptotic endogenous mediators, including the Fas-Fas ligand, tumor necrosis factor (TNF), caspases, and ceramide.6-13 Reactive oxygen species are indeed generated in response to a wide spectrum of apoptotic stimuli and can in turn induce apoptosis in various cells.14,15 Furthermore, the generation of these metabolites with the accompanying intracellular depletion of antioxidants, in particular glutathione, invariably precedes and is associated with apoptosis.16,17 The finding that apoptosis can be blocked by inhibiting or neutralizing ROS further confirms their proapoptotic role.18,19 Among the intracellular...
Table 1. Percentage of Low Levels of DiOC(3)-Positive Lymphocytes From Surgical Patients and Healthy Control Subjects at Each Time Point of the Study*

<table>
<thead>
<tr>
<th>DiOC(3)-Positive Lymphocytes</th>
<th>t₀</th>
<th>t₁</th>
<th>t₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgical patients CD4⁺</td>
<td>26.77 ± 4.57</td>
<td>49.69 ± 7.10†</td>
<td>17.17 ± 4.95</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>19.79 ± 3.10</td>
<td>45.13 ± 4.57†</td>
<td>13.70 ± 2.85</td>
</tr>
<tr>
<td>Control subjects CD4⁺</td>
<td>22.91 ± 3.58</td>
<td>…</td>
<td>…</td>
</tr>
<tr>
<td>CD8⁺</td>
<td>18.74 ± 1.97</td>
<td>…</td>
<td>…</td>
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*Data are given as mean ± SD. DiOC(3) indicates 3,3’-dihexyloxacarbocyanine iodide; t₀, baseline (1 day before surgery); t₁, 24 hours after surgery; and t₂, 96 hours after surgery. †Values are significantly (P<.05) different vs t₀, t₁, and control values.

lar sources of ROS, mitochondria are the most important, and recent evidence has emerged that these organelles also participate in the decision of cells to undergo apoptosis and in the executioner phase of the process. Interestingly, the change in mitochondrial permeability transition that precedes and triggers nuclear apoptosis results in the production of ROS; also, the oxidation-reduction (redox) state in mitochondria is a determinant of apoptosis and may regulate it.

A high degree of oxidant stress, with low levels of endogenous physiological antioxidants, has been demonstrated in subjects recovering from surgery; moreover, some compounds commonly used for inducing or maintaining anesthesia can also affect the metabolism of free radicals. However, to our knowledge, no study has investigated the mitochondrial function in this setting and, in particular, no data are available to support or rule out direct participation of mitochondria in the generation of oxidant stress after surgical trauma and general anesthesia. Based on this background, we tested the hypothesis that perturbations in normal mitochondrial function can be detected in subjects who undergo surgery under general anesthesia. We searched for functional mitochondrial abnormalities that could reveal the cell commitment to apoptosis in samples of peripheral blood lymphocytes taken from a group of surgical patients at different times after the operation. We assayed the mitochondrial transmembrane potential (ΔΨm); the generation of ROS; and the intramitochondrial concentrations of reduced glutathione (GSH), a well-known non-enzymatic cellular antioxidant.

RESULTS

PATIENTS

The total number of patients studied was 16 (9 men and 7 women), and their mean age was 59.2 ± 12.0 years. Types of surgery performed included aortic aneurysmectomy (n = 4), aortic aneurysmorrhaphy (n = 3), hysterectomy (n = 2), bowel resection (n = 4), and partial gastrectomy (n = 3); and the mean duration of surgery was 152.3 ± 35.3 minutes. Intraoperatively, no significant complication related to surgery or anesthesia was observed. No patient was given a homologous transfusion during the study period. The mean total dose of fentanyl was 0.38 ± 0.08 mg. For postoperative pain relief, patients were given our standard analgesic medication, consisting of a combination of ketoprofen and buprenorphine hydrochloride. The mean postoperative hospital stay was 7.92 ± 5.13 days.

MEASUREMENT OF THE ΔΨm

The low incorporation of DiOC₃(3), a cationic lipophilic fluorochrome that allows for the assessment of ΔΨm, is thought to reflect the dissipation of ΔΨm. This change constitutes an early and irreversible step in the effector phase of apoptosis.

Preoperatively (at 1 day before surgery), patients had a low frequency of low levels of DiOC₃(3)-positive lymphocytes that was closely similar to that measured in cells taken from healthy donors. In contrast, at t₁, a relatively high percentage of peripheral blood T lymphocytes incorporated low levels of DiOC₃(3), but at t₂, the rate of lymphocytes with a low level of DiOC₃(3) incorporation returned to values that were similar to preoperative values, being again comparable to that measured in samples taken from the control group. In particular, we investigated separately the incorporation of DiOC₃(3) by CD4⁺ and CD8⁺ lymphocytes. A greater frequency of low levels of DiOC₃(3)-positive cells was measured for both subsets at t₁, and the difference was statistically significant compared with baseline and t₂. Data are summarized in Table 1.

GENERATION OF ROS

We observed that peripheral blood lymphocytes from controls and patients undergoing elective surgery contained a fraction of cells that were able to oxidize the non-fluorescent lipophilic (membrane-permeable) dye HE into the hydrophilic fluorescent product ethidium. Since HE is particularly sensitive to the superoxide anion, this change is thought to reflect the generation of the superoxide anion. Moreover, lymphocytes were labeled using DCFH-DA, a fluorochrome that detects hydroperoxide generation.

We found that the frequency of CD4⁺ and CD8⁺ cells with the HE- and DCFH-DA-positive phenotype was elevated during the early postoperative period compared with preoperative levels. At 1 day before surgery, indeed, surgical patients exhibited a low rate of HE- and DCFH-DA-positive lymphocytes, as this frequency is closely similar to that measured in cells taken from healthy donors. Conversely, at t₁, the frequency of lymphocytes stained with HE and DCFH-DA was significantly higher compared with that at baseline. At t₂, the rate of HE- and DCFH-DA-positive lymphocytes was negligible and returned to values that were similar to preoperative values.

Values of HE- and DCFH-DA-positive CD4⁺ and CD8⁺ cells are depicted in Table 2.

GSH CONCENTRATION

When we recorded the GSH-specific fluorescence of CD4⁺ and CD8⁺ by staining with MBB, we observed that the frequency of cells that stained positive for GSH was...
strongly decreased at t₁ compared with the values measured preoperatively and in control subjects. In turn, at t₂, the rate of CD4⁺ and CD8⁺ lymphocytes stained with MBB was similar to that assessed at 1 day before surgery and that found in the group of healthy controls, indicating that intracellular GSH returned to the concentration detected at baseline (Table 3). Figure 1 and Figure 2 show an example of the original cytofluorometric analysis of mitochondrial function tests.

**LYMPHOCYTE APOPTOSIS**

We measured the frequency of apoptotic CD4⁺ and CD8⁺ lymphocytes in the peripheral blood using flow cytometry after specific staining with 7-amino-actinomycin D and propidium iodide, which allow the recognition of cells undergoing apoptosis, as previously described. The rate of apoptotic cells was significantly (P = .003) increased at t₁ and declined to within the normal range at t₂ (data not shown). This finding indicates that apoptosis is transiently triggered in the early postoperative period, and confirms the results of a recent study in a similar sample of surgical patients.

**COMMENT**

The normal balance between pro-oxidant and antioxidant substances is transiently altered in favor of the former as a result of the surgical trauma and general anesthesia, and the consequent oxidant stress seems to contribute to the pathological injury of tissues observed in several experimental and clinical models of surgical trauma. Furthermore, the plasma levels of oxidized substances have been considered potentially reliable markers of the severity of oxidant stress associated with the surgical trauma.

We performed this study to investigate oxidant stress at the mitochondrial level and to address whether mitochondrial dysfunction is implicated in the increased commitment of lymphocytes to undergo apoptosis that occurs immediately after surgical trauma and general anesthesia. Even though traumatic injury is associated with a greatly enhanced oxidant burst activity of cells, such as neutrophils, no report, to the best of our knowledge, has so far investigated the energy metabolism and oxidant stress at the mitochondrial level in lymphocytes taken from patients undergoing surgery.

In our study, we found a strict association between a pro-oxidant intracellular milieu, characterized by the increased mitochondrial generation of ROS, and the disruption of the ΔΨm, a crucial early step in the process of cellular apoptosis. The phenomenon was transient, as the frequency of lymphocytes with a disrupted ΔΨm was greatly increased at t₁ but returned to the baseline levels and was comparable to control samples at t₂. Also, the increase in the frequency of cells with an enhanced ROS generation was almost transient, and as early as t₂, the frequency of CD4⁺ and CD8⁺ lymphocytes with the HE- and DCFH-DA⁺ positive phenotype returned to the low presurgery basal levels and was not different compared with lymphocyte samples taken from control subjects. Furthermore, this transient alteration in the mitochondrial redox state in favor of a pro-oxidant milieu was associated with a transiently increased commitment to apoptosis. Indeed, the rate of apoptotic CD4⁺ and CD8⁺ lymphocytes was significantly increased at t₁ and returned to within the normal range at t₂, closely paralleling the temporal kinetics of the measured alterations in the mitochondrial redox state. These results are fully in agreement with the findings of a recent study in a similar sample of surgical patients and confirm that commitment to apoptosis is transiently triggered in the early postoperative period.

Our data support the view that oxidant stress and the generation of ROS are probably key factors responsible for the accelerated apoptotic loss of lymphocytes that occurs immediately after surgery. There is, indeed, evidence that cells committed to undergo apoptosis sequentially exhibit a first reduction of the ΔΨm as quantifiable by means of suitable fluorochromes, such as DiOC₆(3), and then an additional decrease in the ΔΨm accompanied by an increased superoxide anion–mediated oxidation of HE into the fluorescent product ethidium. This mechanism is probably a common pathway shared by TNF and a wide range of different proapoptotic stimuli. There is also evidence that mitochondrial dysfunction is implicated in

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<tr>
<th>Table 2. Percentage of HE- and DCFH-DA⁺ Positive Lymphocytes From Surgical Patients and Healthy Control Subjects at Each Time Point of the Study*</th>
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<tbody>
<tr>
<td><strong>Type of Lymphocyte</strong></td>
</tr>
<tr>
<td>Surgical patients</td>
</tr>
<tr>
<td>CD4⁺</td>
</tr>
<tr>
<td>DCFH-DA positive</td>
</tr>
<tr>
<td>Control subjects</td>
</tr>
<tr>
<td>CD4⁺</td>
</tr>
<tr>
<td>DCFH-DA positive</td>
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</tbody>
</table>

*Data are given as mean ± SD. HE indicates hydroethidine; DCFH-DA, 2',7'-dichlorofluorescein diacetate; t₀, baseline (1 day before surgery); t₁, 24 hours after surgery; and t₂, 96 hours after surgery. †Values are significantly (P < .05) different vs t₀, t₂, and control values.

<table>
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<tr>
<th>Table 3. Percentage of GSH-Positive Lymphocytes From Surgical Patients and Healthy Control Subjects at Each Time Point of the Study*</th>
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<td><strong>GSH-Positive Lymphocytes</strong></td>
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*Data are given as mean ± SD. GSH indicates glutathione; t₀, baseline (1 day before surgery); t₁, 24 hours after surgery; and t₂, 96 hours after surgery. †Values are significantly (P < .05) different vs t₀, t₂, and control values.
cellular apoptosis caused by changes in cellular redox potentials and ROS generation, suggesting that mitochondria play a crucial role even in the regulation of the endogenous pathways of apoptosis activated by oxidant stress.16-18 Furthermore, the altered mitochondrial function has been shown to be a better predictor for the initiation of the apoptotic process than the activation of caspase family cysteine proteases that act as crucial endogenous executioners of apoptosis.37

Our hypothesis is reinforced by considering that the temporal profile we observed in the detection of lymphocytes with altered functional activity of mitochondria was closely parallel to that of lymphocyte apoptosis. This observation, along with the experimental demonstration that the detection of cells with a disrupted ΔΨm allows the identification of a subset of cells16-18 that are irreversibly committed to undergo apoptosis despite still lacking the characteristic morphological features of apoptotic cells, adds

Figure 1. Assessment of superoxide anion generation (HE-positive cells) (A and B) and hydroperoxide generation (DCFH-DA-positive cells) (C and D) in peripheral blood T lymphocytes from 2 representative patients. Figures on the left represent values at 1 day before surgery; those on the right, values at 24 hours after surgery; percentages in the upper right, the percentage of cells staining positive; HE, hydroethidine; and DCFH-DA, 2',7'-dichlorofluorescein diacetate.

Figure 2. Assessment of mitochondrial transmembrane potential dissipation (DiOC₆(3)-positive cells) (A and B) and glutathione generation (MBB-positive cells) (C and D) in peripheral blood T lymphocytes from 2 representative patients. Figures on the left represent values at 1 day before surgery; those on the right, values at 24 hours after surgery; percentages in the upper right, the percentage of cells staining positive; DiOC₆(3), 3,3′-dihexyloxacarbocyanine iodide; and MBB, monobromobimane.
further weight to our results and strongly supports the relationship linking the altered mitochondrial function with the commitment of T lymphocytes to apoptosis in patients who undergo surgery under general anesthesia.

Little is still known about the endogenous pathways that are targets for ROS during the process of apoptosis. The proapoptotic activity of ROS may be mediated by several different pathways, such as the activation of nuclear factor κB (NF-κB), a nuclear promoter of TNF production, TNF, the Fas-Fas ligand, caspases, ceramide, and downstream kinases involved in the effector phase of apoptosis; the altered expression of antiapoptotic oncogenes, such as bcl-2; or the decrease in the protective effect of endogenous antioxidants on the expression of proapoptotic genes.6–15

An additional point of interest is that these changes in the mitochondrial redox state that occur early after operation may affect the cell’s vulnerability to inflammation during the postsurgical course. Nuclear factor κB is known to be a redox-sensitive transcription factor, and the mitochondrial redox state appears to be crucial for the transcription of the TNF signal, for NF-κB activation, and for interleukin 6 gene induction.13,30 This suggests that the dysfunction of mitochondria, along with increased oxidant stress and ROS generation, may be a contributing mechanism to the subtle systemic inflammation commonly seen after surgery. On the other hand, the commitment to apoptosis associated with mitochondrial dysfunction could further aggravate the tissue injury caused by inflammation. Whether this could also increase the susceptibility to infection remains to be established. In a previous study, a correlation between the apoptotic loss of the CD8+ population and the risk of infection postoperatively was observed. In the present investigation, we found that 5 patients had a postoperative infection. The only observable difference in the study variables between these patients and those who did not have a subsequent infection was a trend indicating a higher frequency of lymphocytes with low levels of DioC6 (3) in the former group. However, the difference did not reach the level of statistical significance, probably because of the small sample of patients.

We have also observed that the alterations in mitochondrial function were associated with a reduced frequency of lymphocytes that stained positive for GSH on flow cytometric analysis. Although indirectly, this finding probably reflects a significant depletion of the GSH pool at the mitochondrial level. Because mitochondrial GSH is a vital line of defense for metabolizing peroxides, protecting cells against oxygen toxicity,39 the depletion of cellular stores could be an additional factor accelerating the rate of apoptosis triggered by exposure to ROS and oxidant stress.

An important point to speculate on is the position that general anesthesia occupies in the changes of the mitochondrial redox state and in the balance of the process controlling apoptosis following surgery. Recent investigations40,41 have centered on the ability of opioids, compounds used also in anesthesia practice, to trigger apoptosis in several cells, including T lymphocytes.

This apoptotic effect seems to be mediated by specific opioid receptors,42 but alternative sites, such as glucocorticoid receptors, could be involved.43

We are confident that our results, if confirmed, will provide the rationale for designing new practical strategies for the management of patients undergoing surgery and general anesthesia. If oxidant stress would prove a predictor of the risk of postsurgical apoptosis and immune suppression, this would emphasize the clinical sense of giving antioxidant treatment in the perioperative period. However, under the perspective of cost-effectiveness in clinical practice, we urgently need to assess the relative contribution of a wide array of factors, including age, severity of the surgical illness, and comorbidity, to the altered redox metabolism in patients undergoing an operation. Indeed, it should be regarded as more logical to target antioxidant treatment to specific subsets of surgical patients after adjustment for those confounding factors rather than indiscriminately administering ROS scavengers to virtually every subject who undergoes surgery.

To our knowledge, no information is available about whether less invasive surgery, such as laparoscopic surgery, or different regimens of general anesthesia could have less of an impact on the mitochondrial redox metabolism, resulting in a lesser degree of oxidant stress. Previous work has shown that blood levels of the proinflammatory cytokines interleukin 6 and TNF-α are significantly increased in patients undergoing open cholecystectomy compared with subjects undergoing laparoscopic cholecystectomy, but this was independent of the anesthesia regimens used (Surg Laparosc Endosc Percutan Tech. 1999:9:326–332). This finding might indirectly suggest that mini-invasive surgery is associated with a lesser commitment to oxidant stress and apoptosis. Investigation of the potential of mini-invasive surgery and different strategies of anesthesia to trigger apoptosis and immune suppression should be an important point for future clinical research.

Interestingly, Singhal and colleagues44 have observed that catalase, which activates the degradation of hydrogen peroxide in water and oxygen, attenuated the opiate-induced apoptosis in human T lymphocytes. As ROS have been reported to trigger the release of NF-κB,44 these researchers speculated that opiates may act by enhancing lymphocyte expression of NF-κB, which may be mediated through the generation of ROS.42 Furthermore, anesthetics such as isoflurane have the potential to impair the antioxidant defense system.20,27

Since, in our patients, general anesthesia was induced by using fentanyl and isoflurane, we cannot rule out the hypothesis that these compounds could alter directly the redox state in mitochondria and in addition could contribute to the enhancement of lymphocyte apoptosis during the early postsurgical period.

Based on these findings, the clinical potential of antioxidant therapy in this setting should be explored. Antioxidants, such as vitamin E and GSH, prevent NF-κB activation via their antioxidant properties,45 and N-acetylcysteine, another antioxidant that acts as a free radical scavenger, has been shown to decrease NF-κB activation, TNF production, and messenger RNA expression of proapoptotic oncogenes under different experimental conditions.46,47 Further studies are required to estab-
lish whether treatment with antioxidants could be an effective tool under clinical standpoints for counteracting oxidant stress and the increased commitment to apoptosis in surgical patients.

In conclusion, the close link we found in our study between mitochondrial abnormalities and oxidant stress strongly suggests that mitochondrial ROS production may be a major determinant of the lymphocyte apoptosis and immune suppression that follow surgery and general anesthesia.

This study was supported in part by a grant from “La Sapienza” University, Rome, Italy.

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