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SURGICAL ENDOSCOPY
and Other Interventional Techniques

Impact of carbon dioxide versus air pneumoperitoneum on peritoneal cell migration and cell fate

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Abstract
Background: Postoperative systemic immune function is suppressed after open abdominal surgery, as compared with that after minimally invasive abdominal surgery. As a first line of defense, peritoneal macrophages (PMo) and polymorphonuclear neutrophil granulocytes (PMNs) are of primary importance in protecting the body from microorganisms. Previous studies have shown changes in these cell populations over time after open versus laparoscopic surgery. This study aimed to investigate the dynamics of cell recruitment and clearance of peritoneal cells.
Methods: Female NMRI mice (33 ± 2 g) were randomly assigned to carbon dioxide (CO_2) or air insufflation. Intravasal cells with phagocytic capabilities were selectively stained by intravenous injection of the fluorescent dye PKH26 24 h before surgery. Gas was insufflated into the peritoneal cavity through a catheter, and the pneumoperitoneum was maintained for 30 min. Peritoneal lavage was performed 1, 3, 8, or 24 h after surgery. Apoptotic cells were assessed by flow cytometry using a general caspase substrate.
Results: The total peritoneal cell count did not differ between groups. The PKH26-positive PMo level was significantly increased after CO_2, as compared with air, at 1 h and 24 h. The ratio of apoptotic PMo did not differ between the groups. In the peritoneal lavage, polymorphonuclear leukocytes (PMNs) were tripled in the air group, as compared with the CO_2 group, whereas the ratio of apoptotic PMNs was significantly decreased. There was a higher fraction of PKH26-positive PMNs after air exposure, as compared with that after CO_2.
Conclusions: Air exposure triggered a higher transmigration rate of PMNs from the blood compartment into the peritoneal cavity and decreased PMN apoptosis, as compared with CO_2. The lower proportion of PKH26-positive peritoneal macrophages in the air group might have been attributable to a higher inflammatory stimulation than in the CO_2 group, leading to increased emigration of PMo to draining lymph nodes. All the findings underscore a complex cell-specific regulation of cell recruitment and clearance in the peritoneal compartment.

Key words: Apoptosis — Cell migration — Immune function — Laparoscopy — Laparotomy — Peritoneal macrophages — Pneumoperitoneum — Polymorphonuclear neutrophil granulocytes

Minimally invasive abdominal surgery preserves the postoperative local and systemic immune functions better than open abdominal surgery [1, 4, 8, 23]. Animal models have shown increased inflammation [26], impaired delayed-type sensitivity [8, 23, 25], more aggressive metastatic tumor spread [8], and possibly a higher risk of scarring [10] with open surgery.

As a first line of defense, macrophages and polymorphonuclear granulocytes are of primary importance in protecting the body against microorganisms. Macrophages, the most abundant intraperitoneal defense cells, are key effectors and orchestrators of innate immunity. They release several cytokines and mediators to control key events in the initiation and resolution of inflammation. Polymorphonuclear leukocytes (PMNs) are the first blood-borne nucleated cells to populate a site of acute inflammation [27]. However, only upon an inflammatory stimulus do polymorphonuclear granulocytes enter the peritoneal cavity in a significant number and become potent effectors in the first line of defense.

Among leukocytes, PMNs have the shortest half-life [5]. *In vitro*, PMNs die rapidly [9], with the characteristic cell changes for programmed cell death known as apoptosis [21]. The potent intracellular machinery

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responsible for such apoptosis is a family of proteases bearing cysteine at their active site that split their target proteins at specific C-terminal sites of aspartic acids known as caspases (cysteine-dependent aspartate-specific proteases). At inflammatory sites, their constitutive apoptotic program can be modified by different mediators, cytokines, and chemokines. Cells dying in physiologic contexts are rapidly removed by phagocytotic cells such as macrophages [22]. It is widely accepted that macrophages, by phagocytosing apoptotic cells, exhibit an antiinflammatory action [14]. In experimental thioglycollate peritonitis, apoptotic PMNs became engulfed by peritoneal macrophages. However, peritoneal macrophages seem not to undergo apoptosis within the peritoneum, but migrate instead to the draining lymph nodes to apoptose there [2].

Any intraabdominal intervention causes some inflammation, thus provoking an immune reaction. Recently, we were able to demonstrate that all invasive interventions reduced the early postoperative *ex vivo* phagocytosis of peritoneal macrophage (PMo), as compared with sham controls, early after surgery. We also showed that tumor necrosis factor (TNF)-α release by macrophages after *ex vivo Escherichia coli* exposure was diminished in the abdominal air exposure groups, as compared with the carbon dioxide (CO_2) insufflation groups [19]. There is substantial evidence that one difference between groups may be mediated by the biologic effects of the two different gas types. This study therefore focused on two archetypical groups (the air insufflation group and the CO_2 group) with no difference in surgical tissue trauma, but a difference rather in biologic behavior.

Our intentions were to elucidate further the immunologic processes, namely, cell recruitment and resolution of inflammation in our two study conditions. We therefore investigated both the macrophage and PMN migrations from the blood compartment into the peritoneal cavity after air or CO_2 pneumoperitoneum in a murine model. We furthermore studied cell death and determined the apoptosis rate of these cells. We examined different time points in the first 24 h after surgery to assess the kinetics of migration of both the macrophage and PMN into the peritoneal cavity after surgical intervention. Because cell counts differ between study groups over longer periods [12, 24], we studied the dynamics of cell population and their mechanisms. Our working hypothesis was that the pattern of cell recruitment and cell fate alters after different gas exposures of the peritoneal cavity, and that the removal of cells also is changed [2]. We therefore looked for the type of cells and the apoptotic rate of peritoneal macrophages and PMNs present in the peritoneal cavity up to 24 h after surgical intervention.

Materials and methods

Animals

Female NMRI mice weighing 33 ± 2 g were used for all the experiments. The mice were kept free of specific pathogens and allowed *ad libitum* access to food and water throughout the study. All experimental protocols were approved by the local review boards for animal care and in accordance to the Helsinki Declaration. All the animals were allowed to acclimate for a minimum of 7 days before experimental use.

Experimental setting 1

A specific fluorescence marker was intravenously administered to 27 animals 24 h before surgery to label cells selectively with phagocytotic capabilities such as macrophages developed from monocytes or PMNs. By the use of a specific linker technique, dye aggregates or particulates are formed and ingested by phagocytosis *in vivo* or *in vitro* without evidence of functional impairment [16, 17]. This technique allows the quantity of intravascular monocytes that have become peritoneal macrophages within a specified period to be determined [16], as well as the quantity of polymorphonuclear leukocyte granulocytes migrating from the blood compartment into the peritoneum. The highly aliphatic fluorescent dye PKH26-PCL (Sigma-Aldrich, Schnelldorf, Germany; 0.5 µl stock solution in ethanol) was diluted under sterile conditions with diluent B (60 µl; Sigma-Aldrich) to the given *in vivo* labeling concentration of 100 µmol. The mice were fixed in a special fixation cage, and the dye was slowly injected intravenously to a total volume of 100 µl into the mice via the tail vein with a 30-gauge sterile cannula [16].

The mice were randomly assigned to one of the three groups: sham controls undergoing the same anesthesia procedure without any abdominal surgery (control), those undergoing CO_2 pneumoperitoneum (CO_2), and those undergoing air pneumoperitoneum (air). All the mice were anesthetized in an induction chamber using 4% halothane in O_2 and transorally intubated for mechanical ventilation using a wire guide and an operational microscope with 2% isoflurane in O_2 during the whole procedure. Mechanical ventilation was performed using a Hugo Sachs Harvard Apparatus mouse ventilator (Hugo Sachs-Harvard Instruments, March-Hugstetten, Germany) with a tidal volume of 8 µl/g body weight and a frequency of 200 to 240 per minute.

The mice in the sham control group were anesthetized and kept intubated for 30 min. The mice in the CO_2 group were insufflated by CO_2 to a pressure of 10 mmHg using an Olympus laparoscopic insufflator (Olympus UHI-1 high flow insufflator; Olympus Volketswil, Volketswil, Switzerland) through an 18-gauge arterial catheter (Abbocath; Abbott, Sligo, Ireland) inserted into the peritoneal cavity through the right lower abdominal wall. The pneumoperitoneum was maintained for 30 min, after which the abdomen was decompressed and the catheter removed. The mice in the air insufflation group were treated the same way by air insufflation instead of CO_2. All the animals were kept on a warming mat.

The mice were killed 1 h (4 CO_2, 4 air, 3 control) or 24 h (5 CO_2, 5 air, 6 control) after surgery. After they had been killed with CO_2, peritoneal lavage was performed immediately three times with 10 ml of ice cold saline solution through an 18-gauge intravenous catheter (Abbocath-T; Abbott) inserted in the abdominal cavity in the right lower quadrant. All cell isolation steps were performed with the animal on ice or, where not possible, at +4°C.

The cell suspension was centrifuged at 500 g for 12 min to form a pellet. The supernatant was removed, and the cells were resuspended in 1 ml of Dulbecco's modified Eagle's medium (DMEM (Cambrex, Verviers, Belgium)) supplemented with penicillin and streptomycin, but without fetal bovine serum. The cells were counted with a Neubauer hemocytometer, and viability was determined using Trypan blue exclusion. To assess cell differential, cytospins were performed using 50,000 cells in 100 µl DMEM in a Shandon cytocentrifuge at 600 rpm for 8 min, and stained by May-Gruenwald-Giemsa.

Detection of PKH26-stained cells

The percentage of PKH-26-positive cells was analyzed by flow cytometry. Therefore, the PKH26- fluorescence intensity in intact cells was measured. Cells were measured in the flow cytometer (FACScan; Becton Dickinson, Allschwil, Switzerland), and 20,000 counts were recorded for each sample. The fluorescence of PKH26 was detected in channel FL-1. Cells were gated and analyzed to quantify the fractions of PKH26-positive and PKH26-negative cells. These results are shown individually for each cell type, and thus given as percentages. The percentages reflect the actual state of a cell population's fate more clearly (i.e., the fraction directly recruited from blood or the fraction that underwent apoptosis).

Experimental setting 2

To assess cell fate, and assuming apoptosis to be the major pathway of physiologic and pathologic cell elimination in our setting, we determined cellular caspase activity as an established apoptosis marker in 49 animals to elucidate survival of peritoneal macrophages and polymorphonuclear granulocytes. Mice were randomly assigned to one of the three groups and managed surgically as described earlier. The mice were sacrificed 1 h (5 CO_2, 4 air, 3 control), 3 h (5 CO_2, 5 air, 2 control), 8 h (5 CO_2, 5 air, 3 control) or 24 h (4 CO_2, 4 air, 4 control) after surgery. After they had been killed with CO_2, peritoneal lavage was performed, and cells were harvested as described earlier.

Detection of apoptotic cells

To assess the presence of apoptotic cells in the peritoneal lavage, cytospins were stained using a modified method based on terminal deoxynucleotidyl transferase (TdT)-mediated dUTP-digoxigenin nick end labeling (TUNEL) at sites of DNA breaks [7]. Samples were permeabilized with 0.1% Triton-X 100 in phosphate-buffered saline (PBS) for 1 min at room temperature and stained for DNA strand breaks using terminal transferase (Roche, Rotkreuz, Switzerland) incorporating dUTP-FITC (Roche) overnight at 37°C, as previously described [13]. After two 5-min washing steps with PBS, nuclei were counterstained with 1 μg/ml of DAPI (4′,6-Diamidino-2-Phenylindole) in PBS for 10 min. Samples then were embedded with fluorescence mounting medium (DAKO, Zug, Switzerland) and analyzed using an SP2 confocal laser scanning microscope (Leica, Heidelberg, Germany).

Apoptotic cells were quantified by flow cytometry. Therefore, the caspase activity in intact cells was measured with a general cell-permeable caspase substrate [11]. A total of 200,000 cells were stained *ex vivo* with 50 μmol D_2R $(L\text{-}Asp)_2$-rhodamine 110, (Alexis, Lausen, Switzerland). As a pancaspase substrate, D_2R becomes fluorescent only at cleavage by caspases. Among the most commonly activated caspases during apoptosis, D_2R seems to be the best substrate for caspase-3. To differentiate between peritoneal macrophages and peritoneal polymorphonuclear granulocytes, 10 μl of Ly6G-PE antibody (BD Biosciences, Switzerland, Allschwil), which selectively labels granulocytes, was used. Staining with D_2R and antibody was performed for 20 min at 37°C. Cells were measured in the flow cytometer (FACScan; Becton Dickinson, Heidelberg, Germany), and 20,000 counts were recorded for each sample. The fluorescence of D_2R was detected in channel FL-1. The Ly6G-PE-positive polymorphonuclear neutrophils and the Ly6G-PE-negative cells (lymphocytes, macrophages) were assessed in channel FL-2.

Statistical analysis

Analyses were performed with GraphPad Prism, version 3.0 (GraphPad Software, Inc., San Diego, CA, USA) and reported as mean ± standard deviation, or as median and range if not normally distributed,. Normally distributed variables were analyzed by analysis of variance (ANOVA), which was followed, where necessary, by a Bonferroni-corrected post hoc test. All p values of 0.05 or less were considered significant.

Results

Total cell counts differed marginally, but with statistical significance, over time ($p < 0.05$) and between groups ($p < 0.02$). There was no significant difference between the air and CO_2 groups: CO_2 group at 1 h (1.5 ± 0.3 mio cells), at 3 h (1.4 ± 0.3 mio cells), at 8 h (1.6 ± 0.4 mio cells), at 24 h (1.9 ± 0.6 mio cells); air group at 1 h (1.6 ± 0.6 mio cells), at 3 h (1.1 ± 0.1 mio cells), at 8 h (1.8 ± 0.3 mio cells), at 24 h (2.2 ± 0.6 mio cells); and control group at 1 h (1.4 ± 0.2 mio cells), at 3 h (1.0 ± 0.1 mio cells), at 8 h (1.5 ± 0.1 mio cells), 24 h (1.2 ± 0.2 mio cells) ($p < 0.05$). The cell viability in all the groups exceeded 97%, as determined by Trypan blue exclusion.

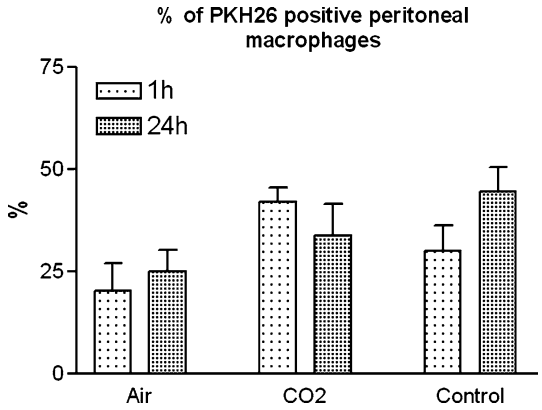

Fig. 1. After air exposure, significantly fewer PKH26-positive peritoneal macrophages were found in the lavage fluid after 1 and 24 h. No difference was found between the carbon dioxide and the control groups.

Experimental setting 1

There was a significant difference in PKH26-positive PMo in the lavage fluid after 1 and 24 h among the groups: CO_2 group at 1 h (42.0 ± 7.2% of total PMo) and at 24 h (25.1 ± 11.5% of total PMo); air group at 1 h (20.4 ± 13.5% of total PMo) and at 24 h (23.2 ± 12.3% of total PMo); and control group at 1 h (30.0 ± 11.0% of total PMo) and at 24 h (44.5 ± 10.6% of total PMo) ($p < 0.05$; Fig. 1). There was a significant difference between the air and CO_2 groups, but no difference was found between the CO_2 and control groups. There also was no difference over time ($p = 0.5$).

The PKH26-positive PMNs also showed a significant difference in cell counts among the groups ($p < 0.04$) and over time ($p < 0.001$; Fig. 2): CO_2 group at 1h (6.9 ± 4.8% of total PMNs) and at 24 h (1.3 ± 3% of total PMNs); air group at 1 h (18.4 ± 10.6% of total PMNs) and at 24 h (7.2 ± 8.1% of total PMNs); and control group at 1 h (26.0 ± 13.2% of total PMNs) and at 24 h (3.6 ± 5.9% of total PMNs). In the post hoc test, the difference between the air and CO_2 groups was significant ($p < 0.03$).

Experimental setting 2

The total number of peritoneal macrophages harvested 1, 3, 8, and 24 h after initiation of the operative procedure significantly differed over the period ($p < 0.03$), but not among the groups ($p = 0.1$): CO_2 group at 1 h (1.1 ± 0.5 mio PMo), at 3 h (1.3 ± 0.3 mio PMo), at 8 h (1.4 ± 0.4 mio PMo), and at 24 h (1.6 ± 0.4 mio PMo); air group at 1 h (1.2 ± 0.5 mio PMo), at 3 h (0.7 ± 0.3 mio PMo), at 8 h (1.2 ± 0.1 mio PMo), and at 24 h (1.6 ± 0.4 mio PMo); and control group at 1 h (1.2 ± 0.3 mio PMo), at 3 h (0.9 ± 0.02 mio PMo), at 8 h (0.9 ± 0.1 mio PMo), and at 24 h (1.2 ± 0.4) (Fig. 3).

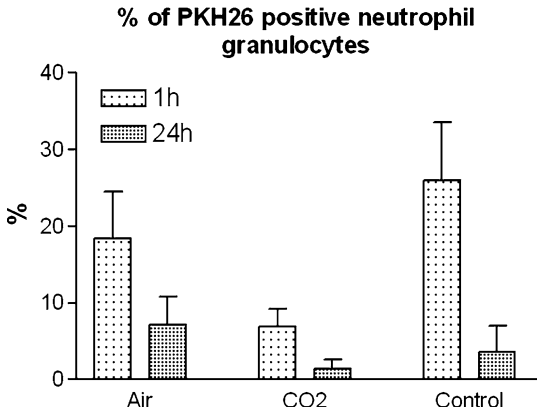

Fig. 2. The PKH26-positive neutrophil granulocyte level was significantly higher in the peritoneal lavage after air than after carbon dioxide (CO_2) pneumoperitoneum. These data suggest a higher recruitment of these cells out of the blood compartment after air exposure than after CO_2 exposure.

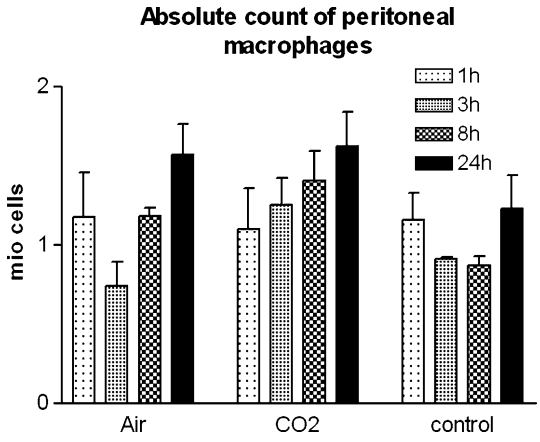

Fig. 3. The number of peritoneal macrophages harvested 1, 3, 8, and 24 h after initiation of the operative procedure showed a significant increase over the period, but there was no significant difference between the groups.

The ratio of active caspase-positive (i.e., D2R- positive) peritoneal macrophages showed no difference between the air and CO_2 groups ($p = 0.71$), but differed significantly over time: CO_2 group at 1 h (25.8 ± 5% of PMo), at 3 h (25 ± 9.7% of PMo), at 8 h (11.1 ± 7.8% of PMo), and at 24 h (29.5 ± 14.4% of PMo); and air group at 1 h (24.7 ± 4.1% of PMo), at 3 h (22.5 ± 12.6% of PMo), at 8 h (11.6 ± 2.8% of PMo), and at 24 h (27.8 ± 10% of PMo) ($p < 0.05$).

The number of polymorphonuclear granulocytes differed significantly both among the groups ($p < 0.01$) and over time ($p < 0.02$) (Fig. 4). There were significantly more PMNs in the peritoneal cavity at each time point in the air group than in the CO_2 group: CO_2 group at 1 h (0.01 ± 0.01 mio cells), at 3 h (0.09 ± 0.08 mio cells), at 8 h (0.12 ± 0.05 mio cells), and at 24 h (0.07 ± 0.08 mio cells); air group at 1 h (0.06 ± 0.04 mio cells), at 3 h (0.18 ± 0.05 mio cells), at 8 h (0.51 ± 0.36 mio cells), and at 24 h (0.13 ± 0.15 mio cells); and control group at 1 h (0.01 ± 0.006 mio cells), at 3 h (0.01 ± 0.01 mio cells), at 8 h (0.02 ± 0.01 mio cells), and at 24 h (0 ± 0 mio cells). Cell differentials performed with flow cytometry

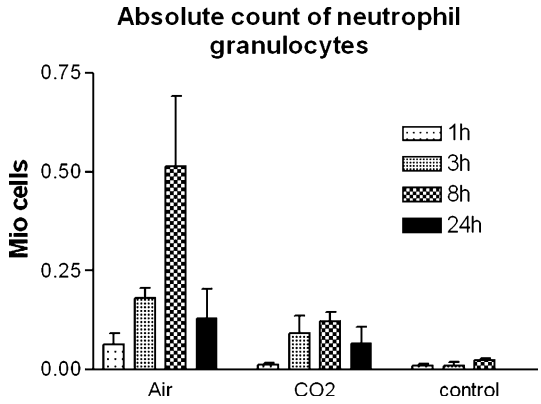

Fig. 4. The number of neutrophil granulocytes differed significantly both between groups and over the period. Significantly more neutrophil granulocytes were found in the peritoneal cavity at each time point in the air group than in the carbon dioxide group.

correlated very closely with the cytospin results, as determined with absolute PMN counts ($r = 0.77$; $p < 0.0005$).

Preliminary results using TUNEL staining clearly demonstrated apoptotic cells in cytospins of the peritoneal cells (Fig. 5). However, there was no way to differentiate between cell types using our techniques, so only the presence of apoptosis could be demonstrated in all three study groups.

The ratio of D_2R-positive neutrophil granulocytes differed significantly both between the CO_2 and air insufflation groups ($p < 0.02$) and over the period ($p < 0.01$; Fig. 6). The ratio of apoptotic neutrophil granulocytes was significantly increased in the CO_2 group, as compared with the air group: CO_2 group at 1 h (23 ± 14% of PMN), at 3 h (43 ± 14% of PMN), at 8 h (11 ± 8% of PMN), and at 24 h (32 ± 26% of PMN); and air group at 1 h (16 ± 8% of PMN), at 3 h (25 ± 8% of PMN), at 8 h (6 ± 2% of PMN), and at 24 h (12 ± 7% of PMN).

There were 134,000 ± 120,000 lymphocytes that did not differ between the groups ($p = 0.9$), nor over the 24-h period ($p = 0.5$). Because the number of D2R-positive lymphocytes was rather small, no conclusive data on lymphocyte apoptosis could be obtained. Mast cells were in the range of 80,000 ± 49,000 cells. No difference was found over time or between groups.

Discussion

The following factors are known to influence postoperative immune status: the operative trauma with its inherent local biochemical alterations such as local eicosanoid, cytokine, and growth factor liberation; hemorrhage and thrombocoagulative processes; cell and tissue edema and destruction; and consecutive reactions that may downregulate such partially inflammatory necrotic or apoptotic cascades [3, 15] and initiate suboptimal or optimal repair mechanisms. We aimed to study whether cell recruitment or cell death could explain these findings, at least in part.

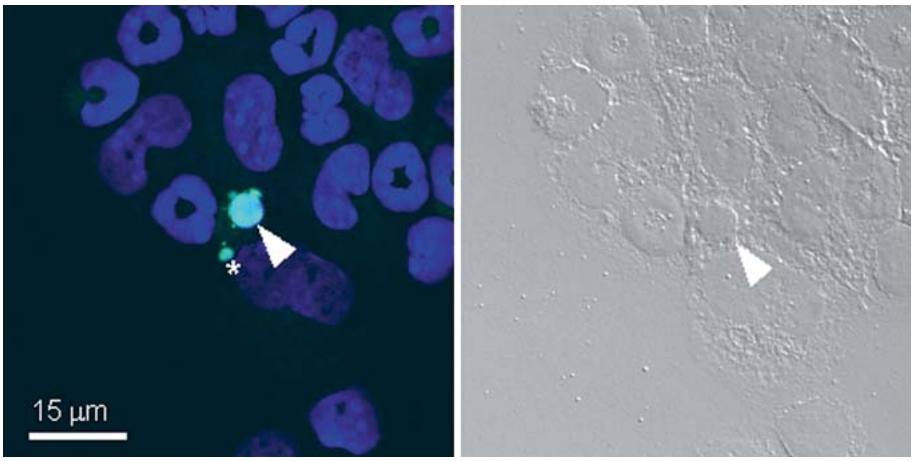

Fig. 5. Morphology of cells after *in situ* nick end labeling (TUNEL) and DNA staining. Cells of a peritoneal lavage were fixed and stained for DNA (DAPI, blue) and TUNEL (DNA strand breaks, green). Low-frequency cells with typical late apoptotic hallmarks such as condensed chromatin (*arrowhead*) allow apoptotic bodies (star) and DNA strand breaks (green) to be recognized. These apoptotic cells often were found engulfed by macrophages.

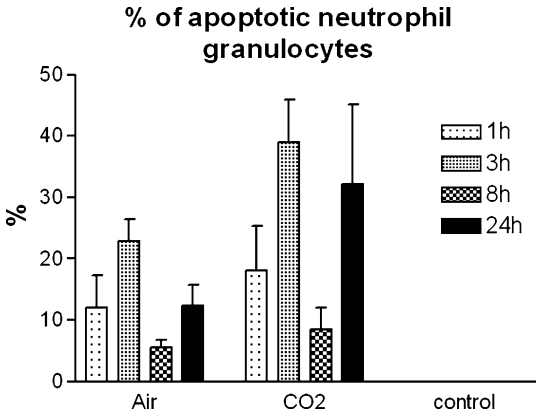

Fig. 6. The ratio of D2R-positive neutrophil granulocytes differed significantly between the carbon dioxide (CO_2) and air insufflation groups. The ratio of apoptotic neutrophil granulocytes was approximately doubled after CO_2 exposure, as compared with air exposure.

In the first 24 postoperative hours, a major difference was observed in both cell distribution and the fate of peritoneal cells between the CO_2 and air insufflation groups. The major incriminated cell type that caused these important differences was PMN, which increased numerically much more in the air group, as evidenced at the 8-h postintervention, in which the difference was approximately a factor of 3, as shown in Fig. 4.

In contrast to the numerically increased PMN in the air group, the apoptosis ratio, as measured using a caspase activity assay, was halved after air exposure, as compared with that in the CO_2 group. Preliminary results using TUNEL staining clearly demonstrated the presence of apoptotic cells on peritoneal cell cytospins. Cell types could not be differentiated by that method. To determine apoptosis within cell populations, fluorescence-activated cell scanning (FACScan) was used.

To assess the dynamics of cells with phagocytotic capabilities, the selective fluorescence marker PKH26 was intravenously injected 24 h before surgery. Such fluorescence-marked PMNs were much more frequent in the peritoneal lavage after air exposure than after CO_2 exposure (Fig. 2). Furthermore, more PMNs also were present intraperitoneally, as evidenced in Fig. 4. The data suggest not only a higher total transmigration rate for PMN, but also, especially, a higher rate of PMN transmigration from the intravascular compartment into the peritoneal cavity in the air group than in the CO_2 group.

As shown in Fig. 2, the most important finding was a significantly higher recruitment over time of PMN from the blood compartment in the air group than in the CO_2 group. However, it must be borne in mind that the absolute PMN count was clearly increased over time in the air group, as compared with the CO_2 group. Together, these findings show that there was more recruitment of PMN from the blood compartment into the peritoneal cavity after air exposure than with CO_2. However, because we have shown less PMN apoptosis in the air group than in the CO_2 group, it might also be speculated that the accumulated cells may have survived longer in the air group than in the CO_2 group. Therefore, the only valid conclusion from the PKH26 results for PMN is that at least one distinct fraction of PMN is recruited from the blood compartment. The data suggest that the pool of PKH26-positive intravascular PMN run out and is replenished from the bone marrow. However we do not have data to prove that this is highly probable.

The peritoneal macrophage numbers did not differ among the groups. Whereas the CO_2 and control groups did not differ in the fraction of recruited cells from the blood compartment, surprisingly, there was a significantly lower percentage of positive peritoneal macrophages in the air exposure group. This finding may reflect either different recruitment sites or fractions other than the blood compartment. Another explanation could be a difference in clearance, such as cell death, be it apoptosis, necrosis, or a combination [6], or emigration of peritoneal macrophages to draining lymph nodes [2]. Because no difference was found in the apoptosis markers of peritoneal macrophages among the groups, apoptosis seems not to be an explanation for different clearance rates. Trypan blue testing at the first centrifugation step underscored the very high viability of cells and no difference among the groups in this or our previous work [19], suggesting that no major amount of late apoptotic or necrotic cells would be Trypan blue positive.

Necrotic cells, on the other hand, could be more difficult to assess than expected due to an inherent instability of their cell structures. A portion of these cells could therefore be lost in the experimental workup during peritoneal lavage, centrifugation, or staining procedures. Therefore, the role of necrotic cells cannot be conclusively determined. Because inflammatory reactions may rather be of a lower grade in our experimental setting, necrosis should not be a major eliminatory pathway for those immune cells. Conversely, emigration to lymph nodes might be a valuable explanation. Bellingan et al. [2] demonstrated that, in contrast to noninflammatory silent peritoneal macrophages that virtually do not move out of the peritoneum, the major mechanism for the removal of inflammatory peritoneal macrophages from the peritoneum during the resolution of inflammation is the process of emigration to the draining lymph node. The time course of this clearance is shown to be very rapid (i.e., within a few hours) [2].

It seems that TNF-α is an important pro-apoptotic factor for PMN in some short time settings of 2 to 4 h, especially in combination with interleukin (IL)-1β [20]. Induction of cell apoptosis may require both macrophage-to-PMN contact and TNF-α presentation on macrophages [18]. However, in 24 to 48 h of TNF-α exposure, its effect seems to be anti-apoptotic [20]. We recently demonstrated a significantly higher *ex vivo* TNF-α release in the air exposure group than in the CO_2 exposure group [19]. However, we did not demonstrate TNF-α present on macrophage membranes. Nevertheless, it seems conceivable that a TNF-α effect of the macrophages may lead to increased PMN apoptosis at earlier time points, as shown in Fig. 6. Certainly, several more pro-apoptocic factors (e.g., FAS ligands, apoptosis-inducing factor) or anti-apoptotic factors such as IL-1β, granulocyte-monocyte colony-stimulating factor, granulocyte colony-stimulating factor, CXC chemokines of the KC- or respective IL-8 group or erythropoietin, and further mediators may be implicated in this highly regulated process of cell death.

In conclusion, peritoneal gas exposure influenced two interlinked factors: cell distribution and cell fate. Air exposure triggered a higher rate of PMN transmigration from the intravascular compartment into the peritoneal cavity, thus increasing PMN numbers. Additionally, decreased PMN apoptosis was found within the first 24 postoperative hours, as compared with CO_2. Both findings corroborate the finding of a higher peritoneal inflammatory status by air exposure than by CO_2 exposure. The significantly lower proportion of PKH26-positive peritoneal macrophages in the air group also may be attributable to a higher stimulation than in the CO_2 group, thus causing increased emigration to draining lymph nodes. All the findings underscore a rather dynamic, complex, and cell-specific regulation of cell recruitment and clearance in the peritoneal compartment.

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