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Kinetics of Decreased LPS-Stimulated Cytokine Release by Macrophages Exposed to CO_2[1]

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The mechanisms responsible for the lack of inflammation after laparoscopic surgery remain unknown. Peritoneal macrophages (MØ) incubated in carbon dioxide (CO_2), but not air or helium (He), had significant, reversible inhibition of lipopolysaccharide (LPS)-stimulated tumor necrosis factor (TNF) and interleukin-1 (IL-1) release. In these experiments the kinetics of these CO_2-induced alterations in cytokine secretion were examined. Murine peritoneal MØ were stimulated with LPS for 4 hr and incubated in different test gases (95% air/5% CO_2, 80% CO_2/20% O_2, 80% He/20% O_2) for intervals between 0.25 and 4 hr. Time between gas incubation and LPS stimulation was varied to determine the persistence of CO_2 inhibition. Parallel MØ groups received LPS stimulation 24 hr later. Supernatant TNF and IL-1 were measured by bioassay and polymerase chain reaction was used to examine cytokine mRNA. Significant reversible inhibition of TNF and IL-1 was seen with CO_2, but not He or air. Inhibition of IL-1 occurred 15 min after CO_2 exposure, was associated with decreased IL-1 mRNA, and was rapidly lost following incubation in the control atmosphere. TNF inhibition was seen despite normal levels of TNF message, required more than 30 min of CO_2 exposure, and persisted after CO_2 removal. CO_2 produced profound, reversible, inhibition of LPS-stimulated cytokine release by peritoneal MØ. The transient inability to secrete inflammatory cytokines after CO_2 exposure may explain the lack of systemic inflammation after laparoscopic surgery with CO_2.

INTRODUCTION

The clinical acceptance of laparoscopic surgery has been overwhelmingly positive. The advantages of laparoscopic cholecystectomy over open operation include: shorter length of stay, decreased post-operative pain, more rapid return to preoperative physical activities, and the absence of intestinal ileus [1–3]. Despite the clinical acceptance and success using laparoscopic procedures, the scientific understanding of these benefits is incomplete. Systemic inflammatory symptoms after major surgery are thought to be produced by host inflammatory mediators produced by many different cells, but particularly macrophages and monocytes. Mediators, such as tumor necrosis factor (TNF) and interleukin-1 (IL-1) can produce hemodynamic alterations, fever, leukocytosis, and even cell injury [4, 5]. Circulating levels of IL-6 were noted to be considerably higher in patients who underwent open cholecystectomy compared to patients who had laparoscopic procedures [6]. In experimental studies the circulating levels of TNF and other mediators were higher after open versus laparoscopic operations; however, the cellular source of the inflammatory mediators was not apparent [7].

We recently reported that macrophages cultured *in vitro* secreted significantly less TNF and IL-1 after lipopolysaccharide (LPS) stimulation if they were incubated in the presence of CO_2 [8]. In those studies no inhibition of cytokine production was seen if macrophages were incubated in air or helium. Furthermore, the CO_2-induced inhibition was not associated with alterations in macrophage viability and was completely reversible [8]. In the present experiments we sought to further investigate the effect of CO_2 on inhibition of LPS-stimulated cytokine release by examining the duration of CO_2 needed to observe this inhibition, the longevity of the inhibited response, and to compare inhibition of TNF and IL-1 gene transcription to gain some insights into the mechanisms involved.

METHODS

Mice. BALBc mice (5–9 weeks old) obtained from Harlan/Sprague–Dawley (Indianapolis, IN) were used for isolation of peritoneal macrophages. Use of animals in these studies was approved by our institutional animal care committee and conformed to the guidelines of the National Research Council. Mice were euthanized with carbon dioxide prior to cell harvest.

Peritoneal macrophage isolation and culture. Peritoneal exudate cells were obtained 72 hr after injecting mice with 2.0 ml Thioglycollate broth (Sigma, St. Louis, MO). Peritoneal exudate macrophages were harvested by peritoneal lavage with 10 ml of Dulbeco's Minimal Essential Medium (DMEM, Gibco, Grand Island, NY) containing 10% calf serum (Hyclone, Logan, Utah) and heparin. Cell viability was determined by trypan blue (Gibco) exclusion and the cells were enumerated with a hemocytometer. Aliquots of 1×10^5 cells in 0.1 ml

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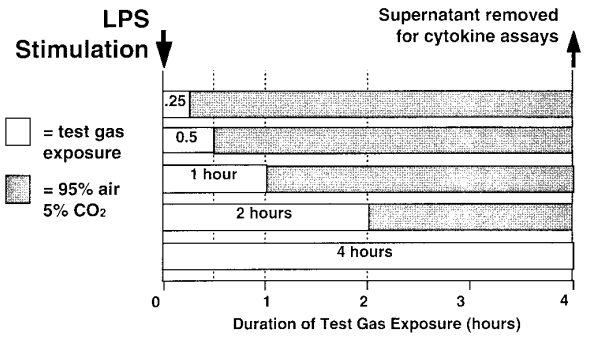

FIG. 1. Schematic depiction of the experimental design used to investigate the duration of test gas incubation on alterations in LPS-stimulated TNF and IL-1 secretion. LPS stimulation was performed for 4 hr in all cases, but the duration of exposure to test gas and control cell culture conditions was varied as shown.

of DMEM containing penicillin (Sigma), streptomycin (Sigma), 2 m*M* L-glutamine (Sigma), and 10% calf serum (Hyclone, Logan, UT) were added to 96-well polystyrene tissue culture plates (Becton Dickinson, Lincoln Park, NJ). The cultures were incubated for 2 hr in humidified air containing 5% CO_2 at 37°C and the non-adherent cells were removed by vigorous washing. The resulting adherent population consisted of >95% peritoneal macrophages (MØ) [9]. The plates were then incubated for 48 hr prior to treatment to allow recovery from the isolation and adherence procedure.

Test gas incubation. Cultured macrophages were exposed to the different test gases by incubation in a sealed chamber, which was flushed with the respective test gas in a modification of a method previously described [10]. Three different incubation gases were investigated; 95% AIR/5% CO_2 (control cell culture atmospheric conditions), 80% CO_2/20% O_2, and 80% helium/20% O_2. All gas mixtures were prepared and certified by the supplier (Minneapolis Oxygen, Minneapolis, MN) and were humidified prior to incubation. The incubation chambers were flushed with the test gas mixtures for a minimum of 5 min after any manipulation. To examine the effect of the duration of test gas exposure the gas incubation was varied as depicted in Fig. 1. Briefly, medium alone or medium containing 1 μg/ml of LPS was added to cultures, the plates were placed in the incubation chambers and immediately flushed with the respective test gases. Cells were incubated for 0.25, 0.5, 1, 2, or 4 hr in the test gas mixtures. At the completion of the designated exposure interval the chambers were opened and the plates transferred to a standard tissue culture incubator with a 95% air/5% CO_2 atmosphere.

In experiments examining the persistence of test gas-mediated alterations the experimental design shown in Fig. 2 was used. All cultures were stimulated with LPS for a duration of 4 hr, but the timing of this stimulation, relative to test gas exposure was altered. The completion of the 2-hr test gas incubation was arbitrarily labeled as timepoint zero. Thus, cultures stimulated with LPS prior to test gas incubation are referred to as time −2 hr. The time points chosen for study were −2, 0, 0.5, 1, and 24 hr after test gas exposure.

In vitro LPS stimulation. Macrophages were activated by exposure to 1 μg/ml of LPS from *Escherichia coli* 011B4 (Sigma, St. Louis, MO). The culture supernatant was discarded and 100 μl of medium containing LPS added. The duration of LPS exposure was 4 hr in all experiments. The timing of LPS exposure varied in individual experiments (see below). At the completion of the LPS stimulation interval supernatant aliquots for TNF and IL-1 bioassays were immediately frozen at −20°C to prevent cytokine deterioration.

TNF bioassay. TNF bioactivity was determined using lysis of L929 fibroblasts (ATCC No. 1-CCL, Rockville, MD) as described previously [11]. Briefly, L929 cells were plated in 96-well plates at 2×10^4 cells/well in 0.1 ml and allowed to grow to near confluence overnight. MØ supernatants were added to the L929 cells in triplicate and serially diluted. The cells were incubated in medium containing 1 μg/ml actinomycin D (Sigma) overnight at 37°C in 95% air, 5% CO_2. To determine the number of viable L929 cells, 50 μl of 2.5 mg/ml MTT dye (Sigma) was added to each well and incubated for 2 hr, after which 100 μl of lysing buffer [20% sodium dodecyl sulfate and 50% dimethyl formamide (Sigma)] was added to each well for 2 hr. The MTT is a dye taken up by the mitochondria of living cells; thus the color released by the lysing buffer is inversely proportional to the amount of TNF present in the sample. The colored product was read at 550 nm in a SLT Model 400 ATC microplate reader (SLT Industries, Salzburg, Austria) and plotted against a standard curve of known concentrations of recombinant murine TNF_α (R&D Systems). Data are expressed as picograms per milliliter TNF.

IL-1 Bioassay. Macrophage production of IL-1_β was determined suing a bioassay with the IL-1 dependent murine T-helper cell line [D10(N4)M; provided by Stephen Hawkins, Cambridge, United Kingdom]. Fifty microliters per well of sample was plated in triplicate on 96-well plates and serially diluted. Aliquots of 1×10^5 D10(N4)M cells/per well were added in RPMI (Gibco) containing 3 μg/ml concon-canvalin A (Sigma) and 1 ng/ml of recombinant murine IL-2 (R&D Systems). The plates were incubated for 72 hr, and then 50 μl of 5 mg/ml MTT dye was added to each well. After 4 hr incubation 50 μl of lysing buffer was added to each well, plates were incubated overnight and the resulting color, proportional to the number of viable cells, was read at 550 nm. Sample IL-1 concentrations, proportional to the number of cells that took up MTT, were determined on the microplate reader at 550 nm by interpolating resultant optic densities against a standard curve of known murine recombinant IL-1_β (R&D Systems) concentrations. Data are expressed as pg/ml IL-1.

Semi-quantitative RT-PCR amplification of TNF and IL-1 mRNA. Total RNA was extracted from cultured macrophages by a modification of the Chomczynski single-step RNA isolation method [12]. For each experimental and control group, mRNA was amplified by reverse transcription with an oligo (dT) primer (Promega, Madison, WI) as described previously [13]. Specific cDNA products corresponding to mRNA for TNF, IL-1, and GAPDH were amplified using the polymerase chain reaction (PCR) [14, 15]. Negative (no cDNA template) and positive (known TNF, IL-1, or GAPDH cDNA sequences) controls were run with their respective reaction mixture. A Model 9600 Perkin–Elmer Thermal Cycler (Perkin–Elmer, Norwalk, CT) was used for amplification with the following sequence profile: initial denaturation at 95°C for 1 min followed by 35 cycles of three temperature PCR (denaturing: 94°C for 45 sec; annealing: 60°C for 45 sec; extension: 72°C for 2 min) ending with a final extension at 72°C for 7 min and cooling to 4°C. Amplified PCR product was identified by electrophoresis of 5- to 10-μl sample aliquots on 2% agarose gel stained with 0.5 μg/ml ethidium bromide. The sample products were visualized by UV transillumination and the gel was photographed. Cytokine cDNA was semi-quantified by densitometric comparison with GAPDH (internal control) from the same sample after the positive image of the gel was digitized by video for computerized densitometry. Data was normalized to GAPDH and is expressed as optical density (O.D.) units.

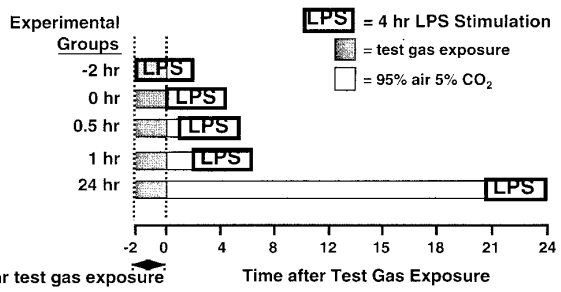

FIG. 2. Experimental design to examine the persistence of test gas-mediated alterations in LPS-stimulated macrophage cytokine secretion. The completion of the 2-hr test gas incubation is arbitrarily defined as the time point 0. All cultures were stimulated with 1 μg/ml of LPS for a duration of 4 hr; however, the timing of LPS stimulation relative to the test gas incubation was controlled as shown. See methods section for complete discussion.

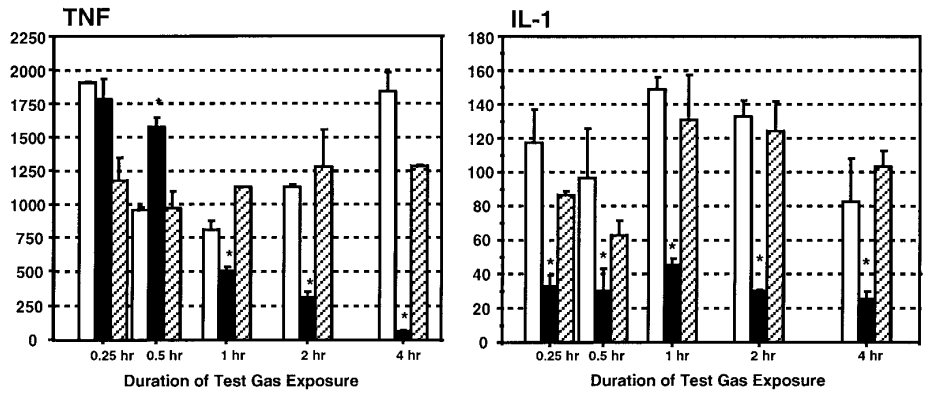

FIG. 3. Effect of duration of test gas exposure on alterations in LPS-stimulated TNF (left) and IL-1 (right) secretion. Cultures were exposed to air (white bars), CO_2 (black bars), or helium (hatched bars) for various times indicated on the *x*-axis. The total duration of LPS stimulation was 4 hr. Cytokine bioassay results are expressed as pg/ml ± SEM. (Key: $*P < 0.01$ versus incubation in air.)

Statistical analysis. Duplicate or triplicate samples were obtained by carrying parallel sets of macrophages through the entire experimental protocol. For the bioassays, each sample was plated in triplicate and the results were averaged. Calculation of sample concentrations by interpolation from standard curves was performed by Deltasoft software (Biometallics, Inc., Princeton, NJ). One-way analysis of variance (ANOVA) and Student's *t* test were used to calculate statistical difference between groups.

RESULTS

Duration of carbon dioxide exposure needed for inhibition of LPS-stimulated cytokine release. The kinetics for CO_2-induced inhibition of cytokine secretion were examined by stimulating with LPS immediately prior to test gas incubation and varying the duration of gas exposure. Because of the time required to complete addition of LPS to cultures and flush the incubation chambers with the humidified test gas mixtures the shortest duration of gas exposure that could be tested was 0.25 hr. Figure 3 shows that CO_2-induced inhibition of IL-1 secretion occurred much more rapidly than inhibition of TNF. Marked inhibition of IL-1 was seen after 15 min of CO_2 incubation, but no inhibition was observed with helium or air. No additional CO_2-induced inhibition of IL-1 protein secretion was seen when the duration of CO_2 exposure was prolonged up to the full 4-hr LPS stimulation interval. In contrast, no inhibition of TNF was seen prior to 30 min CO_2 incubation, but additional duration dependent inhibition was observed with longer incubations. The figure also shows that incubation in air or helium had no significant effects on LPS-stimulated TNF release at any duration of incubation. There was no alteration in macrophage viability following transient exposure to the various test gas mixtures as assessed by trypan blue exclusion or MTT dye release (data not shown).

Persistence of carbon dioxide-induced inhibition of LPS-stimulated cytokine secretion. To investigate the persistence of the CO_2-induced alterations the timing of LPS stimulation relative to the test gas incubation was altered. In all experiments the duration of test gas incubation was constant at 2 hr and the time of LPS stimulation relative to the completion of this incubation was varied. Figure 4 shows that the persistence of CO_2-induced inhibition was different for TNF and

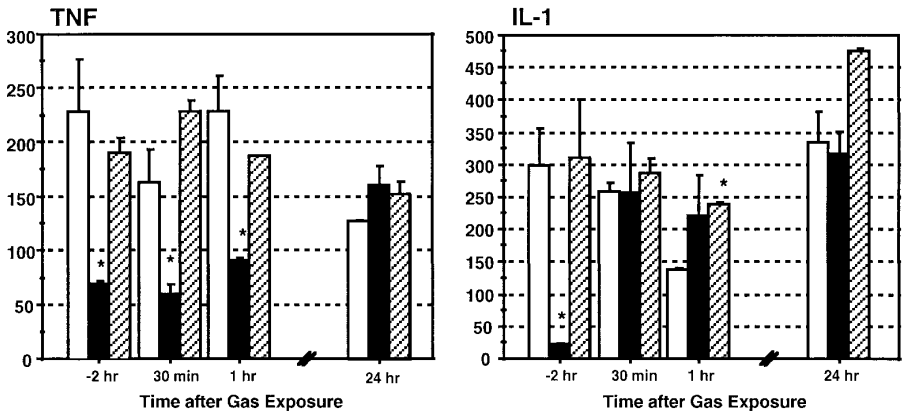

FIG. 4. Persistence of test gas effects on LPS-stimulated TNF (left) and IL-1 (right) secretion in peritoneal macrophages. Test gas incubation groups are as follows: air (white bars), CO_2 (black bars), or helium (hatched bars). Cultures were stimulated with LPS for 4 hr at various times depicted relative to the completion of the test gas incubation. Cytokine bioassay results are expressed as pg/ml ± SEM. (Key: $*P < 0.01$ versus incubation in air.)

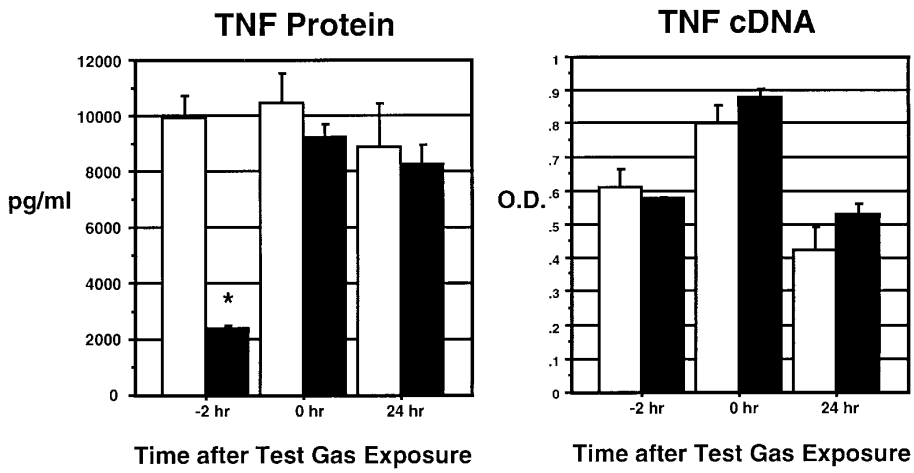

FIG. 5. Comparison of LPS-stimulated TNF protein secretion (left) and cDNA corresponding to TNF message (right) at various intervals relative to test gas incubation. Results of transient incubation in air (white bars) are compared to incubation in CO_2 (black bars). Results for cDNA are normalized to GAPDH as described in the methods section. TNF protein secretion is reported as pg/ml ± SEM and cDNA as arbitrary optical density units ± SEM. (Key: $*P < 0.01$ versus incubation in air.)

IL-1. The most pronounced effects for both cytokines were seen when macrophages LPS stimulation occurred immediately prior to test gas incubation (−2 hr group). When LPS stimulation was delayed until 24 hr after the test gas incubation no inhibition of either cytokine was seen. LPS-stimulated secretion of IL-1 returned to control levels if as little as 30 min elapsed between test gas incubation and stimulation. In contrast, CO_2-induced inhibition of TNF secretion persisted 30 and 60 min after incubation in the control atmosphere. At all intervals examined neither helium nor air incubation produced measurable alterations in cytokine production.

Cytokine mRNA after test gas incubation. We investigated the effects of incubation in air or CO_2 on LPS-stimulated TNF and IL-1 mRNA using RT-PCR to measure specific cDNA 4 hr after LPS stimulation and these results were compared to 4-hr protein secretion by the same macrophages. Figures 5 and 6 show representative results obtained following the 2-hr test gas exposure for TNF and IL-1 respectively. Significant inhibition of both TNF and IL-1 protein secretion was again seen when LPS stimulation was given at −2 hr prior to completion of the gas incubation. In this experiment TNF protein inhibition, shown in Fig. 5, was decreased at −2 hr, but normalized when LPS stimulation occurred at 0 or 24 hr after CO_2. Once again incubation in air did not produce any alterations in TNF secretion. Despite significant inhibition of TNF protein at −2 hr there was no associated alteration in the cDNA for TNF from macrophage cell lysates. Figure 6 shows that there was a significant decrease in the cDNA for IL-1, which corresponded to inhibition of IL-1 protein secretion when LPS stimulation occurred at −2 hr. There was no difference in IL-1 message noted at 0 or 24 hr, which correlated with the absence of IL-1 protein inhibition from the corresponding supernatants. These differences in CO_2-induced alterations in

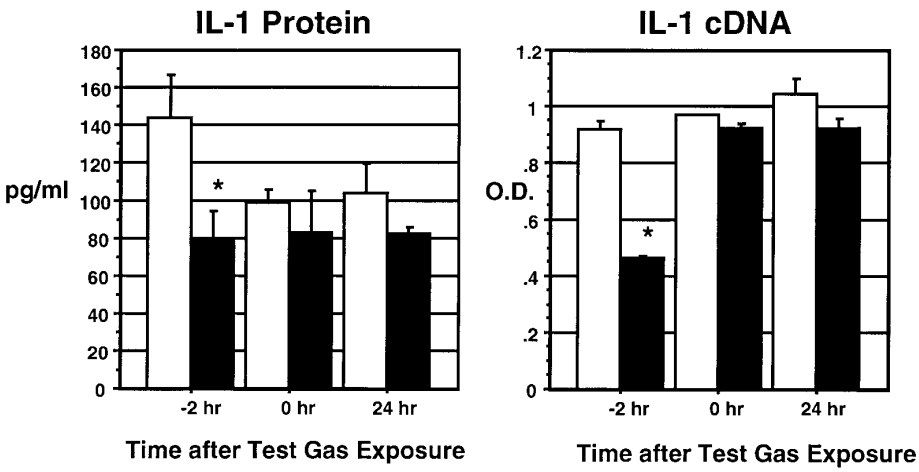

FIG. 6. Comparison of LPS-stimulated IL-1 protein secretion (left) and cDNA corresponding to IL-1 message (right) at various intervals relative to test gas incubation. Conditions and groups are identical to those shown in Fig. 5. IL-1 protein secretion is reported as pg/ml ± SEM and cDNA as arbitrary optical density units ± SEM. (Key: $*P < 0.01$ versus incubation in air.)

cytokine message suggest that CO_2 inhibition of IL-1 and TNF occur via different mechanisms.

DISCUSSION

In these experiments CO_2 incubation resulted in profound, reversible inhibition of LPS-stimulated cytokine production, but the kinetics of CO_2-induced inhibition of TNF and IL-1 were different. Marked inhibition of IL-1 was seen with as little as 15 min of exposure to 80% CO_2/20% O_2, whereas a minimum of 30-min test gas incubation was required to inhibit TNF. In addition, the brief exposure to CO_2 induced near-maximal inhibition of IL-1, whereas the inhibition of TNF was proportional to the duration of CO_2 exposure. Maximal TNF inhibition was not seen until 4 hr of test gas incubation. The capacity of macrophages to secrete both TNF and IL-1 had previously been shown to recover when cells were challenged 24 hr after test gas incubation [8]. In the present studies we found that the CO_2-induced inhibition was quite short-lived. If LPS stimulation was delayed until the completion of the test gas incubation ($t = 0$ hr) the magnitude of inhibition was significantly less and delaying LPS for 1 to 2 hr after test gas incubation essentially abrogated CO_2 inhibitory effects. Finally, qualitatively different patterns of cytokine gene regulation were observed for TNF and IL-1. Messenger RNA for IL-1 was significantly decreased by 50% when CO_2 exposure occurred simultaneously with LPS ($t = -2$ hr) and this was roughly proportional to the magnitude of the inhibition of IL-1 protein synthesis seen at this same time point (142 pg/ml vs 80 pg/ml). In contrast, despite marked inhibition of LPS-stimulated TNF release, TNF mRNA levels were not significantly altered by CO_2 incubation. These results suggest the possibility that CO_2 modulates IL-1 release via a transcriptional control mechanism, while TNF inhibition occurs through a different, post-transcriptional, mechanism.

All experiments were performed in test gas mixtures that contained 20% oxygen to eliminate any confounding hypoxic effects. The viability of macrophages was tested in each experiment using trypan blue exclusion and MTT dye uptake. No alterations in macrophage viability were seen with up to 4 hr incubation in the test gases. Thus, it is unlikely that alterations in viability or hypoxic effects could account for the CO_2-mediated alterations.

Both human and mouse cells respond to LPS with production of cytokines, although human cells make considerably less IL-1 than the mouse cells [16]. Preliminary experiments with human peritoneal macrophages indicate qualitatively similar CO_2-mediated inhibition of cytokine release (M. A. West, unpublished observations). Murine macrophages were investigated because we have a large experience with these cells and the cytokine bioassays used. It is possible that artifacts from using the incubation chambers lead to an underestimate of the degree or rapidity of CO_2-mediated alterations. For example, test gases should rapidly equilibrate with dissolved gas in the culture medium; however, there may be some delay. Because of the time required for addition of culture medium containing LPS the shortest test gas incubation interval examined was 15 min after LPS. Significant inhibition of IL-1 was already apparent after this duration of CO_2, although neither this duration nor 30 min incubation produced TNF inhibition. We cannot say if CO_2 mediated inhibition of IL-1 actually occurs with an even shorter duration of CO_2 exposure. In addition, we chose to perform a 4-hr LPS incubation in all instances. This duration of LPS stimulation is nearly optimal for measurement of TNF protein, but is probably earlier than the peak of IL-1 secretion [4, 5]. It is conceivable that the CO_2-mediated effects were due to a shift in the kinetics of cytokine production. Our method cannot distinguish a profound shift in the kinetics of cytokine production from a decrease in the absolute magnitude of cytokine release; however, based on our familiarity with the murine model system we favor the later explanation.

It was somewhat surprising that inhibition of TNF and IL-1 by CO_2 seemed to occur via separate mechanisms. The findings of different kinetics for CO_2-induced inhibition and recovery from inhibition support this interpretation. In addition, we found that CO_2 had differential effects on regulating LPS-stimulated cytokine mRNA. There are many reports in the literature documenting post-transcriptional regulation of TNF secretion [17, 18]. Thus, the finding of preservation of TNF mRNA is consistent with these reports. However, there have not been previous studies that demonstrated any CO_2-mediated post-transcriptional regulation.

The precise mechanism for CO_2-induced inhibition remains unknown. We reported that extracellular pH was lower for macrophages incubated in CO_2 versus helium or air [8]. It is likely that intracellular acidosis is responsible for inhibition of LPS-stimulated cytokine release, although the intracellular pH has not yet been measured. Macrophages possess a proton pump, which rapidly restores intracellular pH following an acid load, although this pump is ATP dependent the availability of oxygen was not limiting in our experiments [19]. The rapid recovery of LPS-stimulated cytokine production would be consistent with such a mechanism of intracellular respiratory acidosis. Several investigators have demonstrated profound inhibition of human peritoneal macrophage cytokine production when these cells are incubated in an acidic extracellular environment. Mahiout demonstrated that lactate, but not pyruvate, inhibited 24-hr LPS-stimulated TNF_α and $IL\text{-}1_\beta$ secretion by peritoneal macrophages obtained from chronic ambulatory dialysis patients [20]. In a similar study Carozzi *et al.*, showed decreased spontaneous release of IL-1, IL-6, IL-8, and TNF_α when incubations were performed in pH 5.5 medium compared to much higher cytokine levels from cells incubated in medium with a pH of 7.4 [21].

We have not examined whether other macrophage functions are likewise inhibited by CO_2. Specifically, there is no information on whether CO_2 incubation impairs phagocytosis or oxygen radical production. Collet

et al. reported that peritoneal bacterial clearance was greater in animals undergoing laparoscopic operations compared to open procedures and they observed no differences in stimulated oxidative burst between the two groups [7]. On the other hand, Swallow and co-workers showed a 36% decrease in superoxide production by murine peritoneal macrophages when the intracellular pH was pharmacologically decreased from 6.80 to 6.60 [22]. Based on the biochemical pathways involved in oxygen radical generation it is unlikely that macrophages incubated in CO_2 would be able to mount a normal oxidative burst response; however, our results suggest that many of the CO_2-mediated effects are very short-lived. If there was prolonged impairment of peritoneal macrophage host defense functions then patients undergoing laparoscopic procedures should be at increased risk for infectious complications. However, no increase in infectious complications has been observed in large series of laparoscopic cholecystectomies [1], appendectomies [23], or herniorrhaphies [24–25].

In summary, these results describe a potentially physiologic important mechanism of CO_2-mediated alterations in peritoneal macrophage cytokine production, which may partially explain the lack of systemic symptoms after laparoscopic abdominal operations. Our results suggest that different mechanisms exist for CO_2-induced inhibition of IL-1 and TNF, but that any inhibitory effects are very transient. The mechanisms by which local carbon dioxide gas exposure produces alterations of macrophage function are currently under investigation. Understanding how local factors, within the peritoneal cavity, alter the function of resident peritoneal inflammatory cells may have implications for open as well as laparoscopic abdominal operations.

REFERENCES

1. Jatzko, G. R., Lisborg, P. H., Pertl, A. M., Stettner, H. M. Multivariate comparison of complications after laparoscopic cholecystectomy and open cholecystectomy. *Ann. Surg.* 221: 381–386, 1995.
2. Glaser, F., Sannwald, G. A., Buhr, H. J., *et al.* General stress response to conventional and laparoscopic cholecystectomy. *Ann. Surg.* 221: 372–380, 1995.
3. Mealy, K., Gallagher, H., Barry, M., Lennon, F., Traynor, O., Hyland, J. Physiological and metabolic responses to open and laparoscopic cholecystectomy. *Br. J. Surg.* 79: 1061–1064, 1992.
4. Dinarello, C. A. Interleukin-1. *Rev. Infect. Dis.* 6: 51–95, 1984.
5. Dinarello, C. A., Cannon, J. G., Wolff, S. M., *et al.* Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces interleukin-1. *J. Exp. Med.* 163: 1433–1450, 1986.
6. Cho, J. M., LaPorta, A. J., Clark, J. R., Schofield, M. J., Hammond, S. L., Mallory, P. L. Response of serum cytokines in patients undergoing laparoscopic cholecystectomy. *Surg. Endosc.* 8: 1380–1384, 1994.
7. Collet, D., Vitale, G. C., Reynolds, M., Klar, E., Cheadle, W. G. Peritoneal host defenses are less impaired by laparoscopy than by open operation. *Surg. Endosc.* 9: 1059–1064, 1995.
8. West, M. A., Bellingham, J. Carbon dioxide inhibits peritoneal macrophage cytokine production: A mechanism for the lack of inflammatory symptoms after laparoscopic surgery? *Surg. Forum* 46: 147–150, 1995.
9. West, M. A., Keller, G. A., Cerra, F. B., Simmons, R. L. Killed E. coli stimulate macrophage-mediated alterations in hepatocellular function during in vitro coculture. *Infect. Immun.* 49: 563–570, 1985.
10. West, M. A., Li, M. H., Seatter, S. C., Bubrick, M. P. Pre-exposure to hypoxia or septic stimuli differentially regulates endotoxin release of tumor necrosis factor, interleukin-6, interleukin-1, prostaglandin E_2, nitric oxide and superoxide by macrophages. *J. Trauma* 37: 82–90, 1994.
11. Aggarwal, B. B. Human Lymphotoxin. *Methods in Enzymol.* 116: 441–448, 1985.
12. Chomcyznski, P., Sacchi, N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Annal. Biochem.* 162: 156–159, 1987.
13. Seatter, S. C., Bergren, T., Li, M. H., Bubrick, M. P., West, M. A. Macrophage endotoxin tolerance: tumor necrosis factor and interleukin-1 regulation by lipopolysaccharide pretreatment. *Arch. Surg.* 129: 1263–1270, 1994.
14. Mullis, K. B., Faloona, F. A. Specific synthesis of DNA in vitro via a polymerase-catalysed chain reaction. *Methods Enzymol.* 155: 335–350, 1987.
15. Schochetman, G., Ou, C.-Y., Jones, W. K. Polymerase Chain Reaction. *J. Infect. Dis.* 158: 1154–1157, 1988.
16. Seatter, S. C., Li, M. H., Bubrick, M. P., West, M. A. Endotoxin pretreatment of human monocytes alters subsequent endotoxin-triggered release of inflammatory mediators. *Shock* 3: 252–258, 1995.
17. Zuckerman, S. H., Evans, G. F., Gutherie, L. Transcriptional and post-translational mechanisms involved in the differential expression of LPS-induced IL-1 and TNF mRNA. *Immunology* 73: 460–465, 1991.
18. Han, J., Brown, T., Beutler, B. Endotoxin-responsive sequences control cachectin/tumor necrosis factor biosynthesis at the translational level. *J. Exp. Med.* 171: 465–475, 1990.
19. Swallow, C. J., Grinstein, S., Sudsbury, R. A., Rotstein, O. D. Modulation of the macrophage respiratory burst by an acidic environment: The critical role of cytoplasmic pH regulation by proton extrusion pumps. *Surgery* 108: 363–369, 1990.
20. Mahiout, A., Brunkhorst, R. Pyruvate anions neutralize peritoneal cytotoxicity. *Nephrol. Dial. Transplant.* 10: 391–394, 1995.
21. Carozzi, S., Caviglia, M., Nasini, G., Schelotto, C., Santoni, O., Pietrucci, A. Peritoneal dialysis solution pH and Ca^{2+} concentration regulate peritoneal macrophage and mesothelial cell activation. *ASAIO J.* 40: 20–23, 1994.
22. Swallow, C. J., Grinstein, S., Sudsbury, R. A., Rotstein, O. D. Relative roles of Na^+/H^+ exchange and vacuolar-type H^+ ATPases in regulating cytoplasmic pH and function in murine peritoneal macrophages. *J. Cell. Physiol.* 157: 453–460, 1993.
23. Ortega, A. E., Hunter, J. G., Peters, J. H., Swanstrom, L. L., Schirmer, B. A prospective, randomized comparison of laparoscopic appendectomy with open appendectomy. *Am. J. Surg.* 169: 208–213, 1995.
24. Vogt, D. M., Curet, M. J., Pitcher, D. E., Martin, D. T., Zucker, K. A. Preliminary results of a prospective randomized trial of laparoscopic onlay versus conventional inguinal herniorrhaphy. *Am. J. Surg.* 169: 84–90, 1995.
25. Barkum, J. S., Wexler, M. J., Hinchey, E. J., Thibeault, D., Meakins, J. L. Laparoscopic versus open inguinal herniorrhaphy: Preliminary results of a randomized controlled trial. *Surgery* 118: 703–710, 1995.