Peritoneal lymphatic absorption and solute exchange during zymosan-induced peritonitis in the rat

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Carlsson, Ola, and Bengt Rippe. Peritoneal lymphatic absorption and solute exchange during zymosan-induced peritonitis in the rat. Am. J. Physiol. 277 (Heart Circ. Physiol. 46): H1107–H1112, 1999.—Lymph flow is elevated in most inflammatory conditions. However, a few previous studies have indicated that peritoneal lymph flow may actually fall during acute peritonitis. This study was performed to explore this issue further and to study the pathophysiology of peritoneal exchange during peritonitis. Therefore, we wanted to assess the total peritoneal clearance (Cl) and the clearance from peritoneum to plasma (Cl → P) of 125I-labeled albumin (125I-albumin) as well as plasma-to-dialysate clearance (Cl → D) of Evans blue-labeled albumin together with peritoneal ultrafiltration (UF) profiles and mass transfer area coefficients of 51Cr-EDTA and glucose in rats after acute peritonitis induced by zymosan. Zymosan incubation of the peritoneal cavity (120 mg) for 4 h generally led to a 4- to 10-fold increase in peritoneal fluid white blood cell count, indicating that acute peritonitis had been induced. Then 16 ml of 3.86% Dianel and 125I-albumin were instilled intraperitoneally, whereas Evans blue-labeled albumin and 51Cr-EDTA were given as infusions intravenously. Compared with control, mass transfer area coefficients for glucose and 51Cr-EDTA increased markedly from 0.43 ± 0.06 and 0.25 ± 0.04 to 0.91 ± 0.06 and 0.59 ± 0.05 (SE) ml/min, respectively, during peritonitis, whereas Cl and Cl → D increased from 32.8 ± 5.6 and 8.6 ± 1.6 to 74.5 ± 7.3 and 12.9 ± 1.0 µl/min, respectively. The UF profile in peritonitis indicated type I loss of UF (resulting from the increases in permeability-surface area product for glucose). However, the Cl → P declined to 5.9 ± 1.0 µl/min from 7.9 ± 0.8 µl/min (P < 0.05) in control. In conclusion, despite marked effects on peritoneal solute transport and on UF, conceivably resulting from vasodilatation and increases in capillary permeability, zymosan-induced peritonitis did not cause any acute increases in direct peritoneal lymphatic absorption.

lymph flow; peritoneal transport; capillary permeability

DURING TISSUE INFLAMMATION there is normally vasodilation and recruitment of capillaries and at least transient increases in capillary permeability, implying opening of large pores ("gaps") between capillary (venular) endothelial cells. This usually leads to extravasation of plasma proteins and to tissue edema. There may also be changes in the interstitial structure that will contribute to this edema. Data from Østgaard and Reed (13) and Reed and Rodt (15) indicate that an increased negativity in interstitial hydrostatic pressure will enhance the initial fluid extravasation in inflammation. In addition, in nearly all inflammatory conditions, there are marked increases in tissue lymph flow, usually as a consequence of the tissue edema formed, leading to concomitant distension of and fluid propulsion in lymphatics (1).

In clinical peritonitis, there have been a number of reports indicating increases in small and large transperitoneal solute transfer during the acute phase. Furthermore, the increase in large solute transfer has been reported to be far more pronounced than that of small solute transfer. The main problem in clinical peritonitis is, however, that the patients usually arrive at the clinic several hours, or even days, after the onset of peritonitis, which somewhat "blurs" the picture (11). Therefore, there is a need for an experimental approach to study peritonitis under circumstances where tissue inflammation can be induced in a largely controlled fashion.

The most common way to induce peritonitis has been to introduce bacteria intraperitoneally in laboratory animals. The main problem with use of this technique is to titrate out a dose of bacteria that will establish peritonitis without killing the host (2). We chose 4 h of incubation with zymosan A in anesthetized rats to induce peritonitis. Had live bacteria been used in conscious rats, several days would have been required to induce peritonitis, which would have caused pain and suffering for the animals. Zymosan A is a polysaccharide prepared from the cell wall of Saccharomyces cerevisiae. It acts via activation of the complement system, mast cell degranulation, and generation of eicosanoids (7, 9, 24). The induction of peritonitis generally takes place within 30–240 min after the intraperitoneal injection, which implies that peritonitis experiments can be carried out acutely (6, 8).

During peritoneal dialysis (PD) the peritoneum is used as a dialyzing membrane. The transport characteristics of the peritoneal membrane have been described previously at some length (18) and are shown schematically in Fig. 1. Osmotic fluid transport during the first few hours of the dialysis cycle (dwell) is driven by crystalloid osmosis due to the high glucose concentration (76–224 mM) in the dialysate. Solute transport occurs by diffusion, except for macromolecules, which are convected from the blood to the peritoneum or from the peritoneal cavity to the tissues surrounding the peritoneal cavity and to the lymphatics. Thus fluid and solutes are exchanged between the capillaries and the peritoneal cavity but also equilibrate with the tissues surrounding the peritoneal cavity. The subdiaphragmatic lymphatic system seems to be the most important pathway for lymph drainage from the peritoneal cavity. However, lymphatics in the visceral and parietal parts of the peritoneum also contribute. It has been known for almost two decades that the clearance of a...
METHODS

Seemed to decline slightly. Absorption did not increase during peritonitis; rather, it other tissues during inflammation, "direct" lymphatic drainage from the peritoneal cavity. We were surprised to find that, unlike the situation in most other tissues during inflammation, "direct" lymphatic absorption did not increase during peritonitis; rather, it seemed to decline slightly.

**Fig. 1.** Transport of small solutes (bidirectional diffusion, dotted arrows) and fluid (thin arrows) and macromolecules (convective clearance, thick arrows) between peritoneal cavity and interstitial space. Transport to large diaphragmatic stomata takes place in bulk directly from peritoneal cavity. Regarding visceral and parietal lymphatic pathways, there may be some degree of size discrimination due to impact of interstitial space. There is no direct backdiffusion of albumin into capillaries, which means that amount of 125I-labeled human serum albumin (RISA) detected in plasma is largely due to lymphatic drainage from peritoneal cavity.

macromolecular tracer, placed in the peritoneal cavity, will be much higher (during the first few days of tracer equilibration) than its clearance by the lymphatics into the plasma. The latter seems to be the best "indirect" measure of peritoneal lymphatic drainage (21).

The present experiments were performed to investigate the pathophysiological alterations of transperitoneal exchange during the initial acute phase of peritonitis. A major aim was also to monitor changes in lymphatic drainage from the peritoneal cavity. We were surprised to find that, unlike the situation in most other tissues during inflammation, "direct" lymphatic absorption did not increase during peritonitis; rather, it seemed to decline slightly.

**METHODS**

Experiments were performed on 21 male Wistar rats (Møllegaard, Copenhagen, Denmark; B & K Universal, Sollentuna, Sweden) having an average body weight of 244.9 ± 79.2 (SD) g and allowed free access to food and water until the day of the experiment. Anesthesia was induced by an injection of pentobarbital sodium (50 mg/kg body wt ip) and maintained by repeated intra-arterial administration of the same drug (~15 mg/kg).

The rats were placed supine on a heating pad. The tail artery was cannulated for continuous blood pressure monitoring on a polygraph (Grass Instruments, Quincy, MA) and for administration of drugs. A tracheotomy was performed to facilitate breathing. The left carotid artery was cannulated for blood sampling, and the jugular vein was cannulated for infusions of saline and ⁵¹Cr-EDTA. Access to the peritoneal cavity was obtained via a catheter (Venflon Ø, 1.7 mm, BOC Ohmeda, Helsingborg, Sweden) placed in the lower left part of the abdominal wall. The animals were left for ≥20 min after surgery to stabilize.

The peritonitis group (n = 8) was treated with zymosan (15 mg; Sigma Chemical, St. Louis, MO) diluted in 2 ml of PBS for 4 h, and an incubated control group (n = 7) was given PBS (2 ml) 4 h before the dwell (dialysis cycle). Dialysis was then performed using 3.86% Dianeneal (Baxter Health Care, Castlebar, Ireland). Control experiments without incubation (n = 6) were carried out using the same method as in the other experiments, except the PD dwell was started directly 20 min after surgery.

⁵¹Cr-EDTA (0.09 MBq in 0.2 ml; Amersham Life Science) was given as a bolus dose intravenously at the start of the dialysis dwell and then as a constant infusion at 1.2 ml/h (0.22 MBq/ml). The plasma level of ⁵¹Cr-EDTA was kept constant, and the tracer appearance in dialysis fluid was measured to obtain the permeability-surface area product (PS, mass transfer area coefficient (MTAC) of ⁵¹Cr-EDTA (3)). BSA (fraction V solution, Sigma Chemical) was labeled with Evans blue (Sigma Chemical) and given in the tail artery cannula (0.8 ml of 12 mg/ml Evans blue in 10% BSA-saline) to assess albumin clearance from plasma to dialyse (Cl → D).

Dialysis was performed using 16 ml of 3.86% Dianeneal containing 100 µl of 17.5% BSA (Sigma Chemical) and 125I-labeled human serum albumin (RISA, 0.1 MBq; Isoharma, Kjeller, Norway) as an intraperitoneal volume marker to calculate the total RISA clearance from the dialysate (CI) and the dialysate-to-plasma clearance (CI → P) of RISA [as a marker for peritoneal lymph flow (28)]. After 120 min the dialysate was totally recovered using a syringe and preweighed gauze tissues. After the dwell the peritoneal cavity was rinsed with 19 ml of 3.86% Dianeneal. All solutions were prewarmed to 37°C before instillation.

Two 25-µl blood samples were taken before and at 5, 10, 20, 40, 60, 90, and 120 min of the dwell. Before the experiment and at 60 and 120 min an additional 400 µl were taken for glucose determination. Hematocrit was measured in the beginning, after 60 min, and finally at the end of the experiment. Dialysate sampling was done at 5, 10, 20, 40, 60, and 90 min of the dwell and from the drained fluid and also from the rinsing fluid; two 25-µl samples were taken for radioactivity measurements, and in addition, 80 µl were collected for absorbance and glucose measurements. Radioactivity was measured using a gamma counter (Wizard 1480, LKB-Wallac, Turku, Finland). Glucose concentrations in plasma and dialysate were measured in an automatic analyzer (model ABA100, Abbott) with use of hexokinase and glucose-6-phosphate dehydrogenase reagent (Glucose-UV, Abbott). Plasma samples for absorbance assessments were diluted in 150 µl of saline before measurement. Absorbance in dialysate and plasma samples was measured at 620 nm by use of a spectrophotometer (Spectronic Genesys 5, Milton Roy, Rochester, NY). White blood cells (WBC) in the intraperitoneal fluid were counted after incubation in three control rats, three incubated control rats, and three zymosan-treated rats.

Calculations were performed as previously described (3, 18). PS for glucose and ⁵¹Cr-EDTA were averaged from sequential measurements throughout the dwell. Dialysate volume was extrapolated from measured intraperitoneal RISA concentration values with the assumption that 4–5% of RISA binds to the peritoneal linings (26) (indicator-dilution...
RESULTS

The number of WBC in the intraperitoneal fluid before instillation of dialysis fluid in control rats was not different from that in incubated controls (2,574.5 ± 796 vs. 3,430 ± 557 cells/mm³, not significant) but increased significantly in the peritonitis group (11,950 ± 1,934 cells/mm³) vs. the two control groups (P < 0.05). There was also a shift from mononuclear cells toward polymorphonuclear cells during peritonitis (Fig. 2).

Incubation of animals increased the “residual” intraperitoneal volume compared with nonpreincubated animals, yielding a higher starting point of the volume vs. time curves. The residual intraperitoneal volume in peritonitis (5.53 ± 0.35 ml) was not different from that in incubated controls (4.19 ± 0.38 ml), whereas it was only 2.29 ± 0.50 ml (P < 0.01 and P < 0.001, respectively) in controls (Fig. 3). With the initial intraperitoneal volume set at 100%, there was a marked change in the peak time of the volume vs. time curve during peritonitis, the peak time being only 40 min compared with 90 min in incubated controls. During control, peak volume was reached first after 2 h of dwell (Fig. 4). In peritonitis there was a significantly increased ultrafiltration (osmosis) during the initial part (5–20 min) of the dwell compared with the situation in control (P < 0.001) or incubated control rats (P < 0.01). At the end of the dwell (120 min) there was a significantly higher intraperitoneal volume in controls than in incubated controls (P < 0.01) or in the peritonitis group (P < 0.01). However, there was no difference among incubated control rats and the peritonitis group (Fig. 4).

The PS or MTAC for 51Cr-EDTA, as averaged from sequential measurements for the whole dwell, was significantly increased in the peritonitis group compared with control rats: 0.586 ± 0.050 and 0.250 ± 0.038 ml/min, respectively (P < 0.001). Incubated controls, however, showed a PS for 51Cr-EDTA comparable to that in controls: 0.347 ± 0.031 ml/min (not significant). There was a significant difference between incubated controls and the peritonitis group (P < 0.01; Fig. 5A). Also, PS for glucose increased markedly in the peritonitis group (0.914 ± 0.062 ml/min) compared with control rats (0.433 ± 0.056, P < 0.001). PS for glucose in incubated control rats was also significantly different from that in controls (0.728 ± 0.071 ml/min, P < 0.01), but there was no difference between incubated controls and the peritonitis group (Fig. 5B).

The clearance of Evans blue-labeled albumin from plasma to dialysate (Cl → D) was markedly increased in the peritonitis group (12.94 ± 1.04 µl/min, P < 0.05 vs. controls) but unchanged in the incubated control group compared with controls (8.18 ± 0.56 vs. 8.57 ± 1.58 µl/min, not significant; Fig. 6A).

The clearance of RISA out of the peritoneal cavity (Cl) in incubated control rats was not significantly changed compared with controls (46.37 ± 4.91 vs. 32.81 ± 5.55 µl/min) but markedly increased in the peritonitis group (74.46 ± 7.25 µl/min, P < 0.001 vs. controls and P < 0.01 vs. incubated controls; Fig. 6B).

Lymph flow from the peritoneal cavity, measured as RISA clearance to plasma (Cl → P), remained low in all groups and actually seemed to decrease during peritonitis compared with incubated control rats: 5.95 ± 1.03 vs. 8.96 ± 0.81 µl/min (P < 0.05; Fig. 6C).

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V(t) = a + b \cdot \left[ 1 - \exp(-c \cdot t) \right] - d \cdot t
\]

where \( V \) is volume, \( a, b, c, \) and \( d \) are parameters, and \( t \) denotes time (independent variable). This allowed us to calculate a volume at \( t = 0 \) from the parameter \( a \) (apparent intraperitoneal volume at \( t = 0 \)) minus the given volume of dialysate.

Values are means ± SE. Statistics was obtained using one-way ANOVA and post hoc tested using Bonferroni’s correction. All statistical calculations were made using the computer program SPSS for Macintosh (release 6.1.1, SPSS, Chicago, IL).
DISCUSSION

During peritonitis there was an increase in the transport of small and large solutes between plasma and dialysate (11, 22). These changes may be explained by a vasodilatory recruitment of capillaries in the peritoneal membrane and an acute opening of large pores in the microvessels (mainly in postcapillary venules) caused by inflammatory mediators and cells. There may also be changes in the structure of the interstitium contributing to the increases in solute transport (16).

As a sign of acute peritonitis in the present study, a major flux of polynuclear WBC into the peritoneal cavity occurred in the peritonitis group that was not seen in controls or in incubated controls. Injection of anesthetics into the peritoneal cavity may have irritated the tissues, causing some increases in WBC also in controls. In peritonitis there was, however, a shift toward polynuclear cells associated with the increases in WBC count. This indicates that a true inflammatory reaction may have occurred in the peritonitis group. Indeed, other groups have also been able to show similar increases in WBC count after, e.g., Escherichia coli infections in rats (14). Furthermore, Korybalska et al. (10) showed that treatment with lipopolysaccharide, a well-known mediator of WBC activation, given in the dialysis fluid, increased the WBC approximately fourfold compared with the control situation.

There was an increased "resting" intraperitoneal volume in the peritonitis group because of the inflammatory process, which was actually also to some extent seen in the incubated control group. This suggests that there may, after all, have been some irritation caused by the injection of the PBS per se and/or by the intraperitoneal anesthetics. However, the intraperitoneal volume in the peritonitis group was indeed significantly increased compared with that in incubated controls.

The transperitoneal glucose osmotic gradient declined more rapidly in the peritonitis group than in the controls and the incubated controls, as evidenced by the markedly increased PS for glucose during peritonitis. PS for $^{51}$Cr-EDTA and for glucose are assumed to increase during peritonitis mainly because of recruitment of capillaries, as seen in the present study. Actually, because of increases in surface area ($S$) during peritonitis, there was also an increased osmosis (ultrafiltration), conceivably also due to an increased hydraulic conductance established during the first 20 min of the dwell with an earlier ultrafiltration peak. However, had hydraulic conductance and PS for glucose been increased to the same extent (via $S$), there would have been an increased ultrafiltration curve peak height in the peritonitis group, which was not seen (19). Hence, the permeability for glucose must have been increased separately from $S$, making the changes in PS for glucose larger than those in hydraulic conductance.

Macromolecular exchange across capillaries seems normally to occur in a unidirectional fashion, i.e., more or less from blood to interstitium and not vice versa (17). Thus the increased clearance of albumin out of the peritoneal cavity (CI) during peritonitis and in incubated controls cannot be explained on the basis of microvascular changes per se. Rather they seem to reflect alterations in the interstitium, conceivably via an altered interstitial structure. Already during ordinary PD dwells, there is evidence of some edema formation in the tissues surrounding the peritoneal cavity (27). However, this slight edema may be greatly enhanced during conditions of inflammation because of an increased negativity of the interstitial fluid (13), further reducing the tissue resistance to intraperitoneal colloid permeation in peritonitis.

Two previous studies have suggested that peritoneal clearance of macromolecules to plasma "lymph flow" may actually decline during peritonitis. In these studies, lymph was collected by direct cannulation of efferents from the caudal mediastinal node and of the thoracic duct, combined with tracer appearance experi-
ments (20, 25). The results from these studies are in line with the results of the present study, at least regarding the difference in Cl → P between incubated controls and rats with peritonitis. Reductions in Cl → P may have occurred because of the large amount of WBC in the dialysate, which may, at least partially, hamper flow through the lymphatics. Such effects may have a major impact on the diaphragmatic lymph flow, which accounts for a large portion of the lymphatic drainage from the peritoneal cavity (28). Thus there are wide stomata in the diaphragm that drain the peritoneal cavity and may be partially blocked by cells. There may also be a reduction of lymph flow due to inhibitory action on lymphatic pumping by inflammatory mediators released from monocytes (23). Some authors have also discussed the possibility that reduced respiratory movements, and thus a decreased lymph propulsion in diaphragmatic lymphatics, may inhibit lymphatic drainage when the intraperitoneal volume is increased during peritonitis (12). In our study the intraperitoneal volume was unaltered among controls and the peritonitis group, so there must be other reasons for the slight reduction in lymph flow. Rather, lymph flow may have been reduced because of the presence of intraperitoneal inflammatory mediators or the large amount of WBC present interstitially during peritonitis.

In summary, the transport alterations during zymosan-induced peritonitis show a complex pattern. During the initial phase of peritonitis, there was a major

Fig. 5. A: mean permeability-surface area product (PS) for 51Cr-EDTA averaged for whole dwell. B: PS for glucose. **P < 0.01 and ***P < 0.001.

Fig. 6. A: clearance to dialysate of Evans blue-labeled albumin. B: total clearance of RISA from dialysate. C: direct lymphatic absorption, measured as clearance of RISA from dialysate to plasma. *P < 0.05, **P < 0.01, and ***P < 0.001.
efflux of plasma proteins and WBC into the peritoneal cavity, concomitant with marked increases in large and small solute transport, conceivably due to precapillary vasodilatation and recruitment of capillaries and marked increases in vascular permeability. Also, albumin entrance from the peritoneal cavity into tissues surrounding the cavity increased notably, indicating that interstitial alterations accompany peritonitis. However, despite large changes in the majority of exchange parameters measured, albumin clearance to plasma, which has been shown to be a good measure of direct peritoneal lymphatic absorption, was not significantly increased from control. Rather, it seemed to have declined slightly. Why peritoneal direct lymphatic absorption remains unchanged or even declines during peritonitis, in stark contrast to the lymph flow enhancement in most other inflammatory conditions, is intriguing and deserves further study.

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REFERENCES