

LYMPH FLOW AND LYMPHATIC DRAINAGE OF INFLAMMATORY CELLS FROM THE PERITONEAL CAVITY IN A CASEIN-PERITONITIS MODEL IN SHEEP

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ABSTRACT

The purpose of this study was to characterize the cellular responses in the peritoneal cavity and draining lymph in a sterile peritonitis model in conscious sheep. Lymph was collected from lymphatics that drained the peritoneal space (caudal mediastinal and thoracic ducts) as well as from lymph vessels that drained peripheral tissues (prescapular). Casein was used as the inflammatory agent. Dialysis solution (Dianeal® 4.25%) containing 1g% casein and 25 µCi ¹²⁵I-human serum albumin was infused into the peritoneal cavity in 50 ml/kg volumes. Peritoneal volumes increased from a mean infused volume of 1572±51 ml to a maximum of 2119±77 ml at 3 hours. Over 6 hours, the number of macrophages and lymphocytes in the peritoneal cavity remained relatively constant but the number of neutrophils increased from $9.9 \pm 4.2 \times 10^7$ to $9.2 \pm 1.9 \times 10^9$ total cells. Caudal lymph which drains directly from the peritoneal cavity through diaphragmatic stomata, demonstrated a 5 fold increase in flow rate over 6 hours following the Dianeal-casein infusion. Thoracic duct and prescapular flows declined approximately 70% and 50% respectively in the same time period. The concentration of lymphocytes and the lymphocyte outputs (product of volume and concentration) declined in all lymph compartments. No elevations in

neutrophil numbers in the thoracic and prescapular lymph compartments were observed but neutrophil output in the caudal lymph increased steadily from $3.1 \pm 1.5 \times 10^6$ to $4.6 \pm 1.3 \times 10^7$ /hr at the 6 hour mark. We conclude that the major route of removal of inflammatory cells and fluid from the peritoneal cavity is through diaphragmatic lymphatics.

In peritoneal dialysis, large volumes of hypertonic dialysis solution are infused into the peritoneal cavity. Lymphatic vessels remove a portion of this dialysate and as a result, peritoneal lymph drainage is increasingly under investigation as a factor that may contribute to ultrafiltration failure in some patients on chronic peritoneal dialysis (1-4). Another potential cause of ultrafiltration failure is recurring infection in the cavity. Peritonitis is due primarily to infection with *S. epidermidis* or *aureus* (5,6) and is the most frequent complication in these patients. Ultrafiltration volumes are significantly reduced during episodes of infection (7). At present, ultrafiltration failure necessitates transfer to hemodialysis which has a greater impact on the health care system in terms of limited hemodialysis facilities and cost.

Since lymph flow rates have been demonstrated to increase in many types of inflammatory reaction, one might expect that the

same would be true of the peritoneal cavity and that increased lymph drainage may contribute to reduced ultrafiltration. In humans, the clearance of macromolecules from the peritoneal cavity is increased during peritonitis (8). Alternatively one might argue that inflammation might reduce lymph drainage of the peritoneal cavity. Fibrin and platelet aggregates formed during an inflammatory event, may occlude the stomata which are the major structures linking the peritoneal cavity with afferent diaphragmatic lymphatic vessels. Dumont et al (9) demonstrated that the intraperitoneal injection of platelets decreased diaphragmatic absorption of lipid and carbon particles from the peritoneal cavity. Wilcox et al (10) demonstrated that monocyte-derived inflammatory mediators inhibited contractions of the diaphragm. Since diaphragmatic movement contributes to the transport of fluid from the cavity into lymphatics (11), one might argue that certain inflammatory mediators reduce lymph drainage of the peritoneal cavity. In any event, lymphatic drainage of the peritoneal cavity may be significantly altered in peritonitis. The lymphatics may not only facilitate the loss of ultrafiltration but also contribute to the dissemination of the infectious agent or endotoxin into the bloodstream (12). In colonic perforation experiments, Dumont et al (13) demonstrated that injection of platelet rich plasma or scarification of the peritoneal surface of the diaphragm increased survival time in rats by blocking fecal material uptake into lymphatics.

As ultrafiltration failure has been linked to lymphatic drainage, and peritonitis with ultrafiltration failure, the potential relationship between inflammation and peritoneal lymph drainage appears to warrant further investigation. As a first step, the purpose of this study was to develop a peritonitis model in sheep and characterize the cellular responses in the peritoneal cavity and draining lymph. The sheep was chosen as the experimental model since peritoneal lymphatic drainage pathways are known (14), and

several of these are accessible to cannulation (15)

MATERIALS AND METHODS

Animals

Five randomly bred female sheep, ranging in weight from 28 to 34 kg (mean 31.2±1.1) were used in this investigation.

Anesthesia and Surgery

Pentothal sodium (Boehringer Ingelheim, Burlington, Ontario, Canada) was given intravenously (15 to 25 mg/kg body weight) to induce anesthesia. The sheep were then intubated with a Sheridan/HVT tracheal tube (Sheridan Catheter Corp., Argyle, NY, USA). Anesthesia was maintained with 1 to 2% fluothane (Halothane, Ayerst Laboratories, New York, New York, USA) in oxygen using a respiration pump (Ventimeter Ventilator, Air Shields Inc., Hatboro, Pennsylvania, USA).

A small right lateral incision was made approximately 10 to 12 cm posterior to the last rib through which a Tenckhoff peritoneal dialysis catheter equipped with two Dacron cuffs (Accurate Surgical Instruments Corp., Toronto, Ontario, Canada) was inserted and the wound carefully closed with silk suture. Also at this time, a catheter was placed in the right external jugular vein for sequential blood sampling.

The efferent lymphatic ducts of the caudal mediastinal lymph node and the prescapular lymph node were cannulated as described in our previous papers (15-18). The right lymph duct was left intact. A 5 to 6 cm length of the thoracic duct was cleaned of connective tissue and fat. A plastic catheter was inserted against the direction of flow and tied in place with silk suture. The outflow end of the catheter was inserted 3 to 4 cm downstream into the thoracic vessel in the direction of flow such that thoracic duct lymph continued to flow into the catheter but was returned to the thoracic vessel. The loop of thoracic catheter

was externalized and tied to the sheep's wool until the following day. At the start of the experiment, the loop of catheter was cut and thoracic duct lymph collected for the duration of the experiment. This procedure prevented the significant losses of protein, liquid and lymphocytes that would have occurred if thoracic duct lymph was diverted from the sheep for the 24 hour recovery period.

All sheep were allowed to recover for 24 hours in a metabolism cage before experiments were performed.

Tracers and Solutions

Hypertonic dialysis solution (Dianeal® 4.25%) was obtained from Baxter Healthcare Corporation (Deerfield, IL). Casein (sodium salt) was purchased from Sigma (St. Louis, MO) and ¹²⁵I-human serum albumin solution (2.5 µCi/mg, 10 mg albumin/ml), purchased from Frosst (Kirkland, Quebec).

Experimental Protocol

Via the peritoneal catheter, 50 ml/kg body weight of prewarmed (37°C) Dianeal 4.25% containing 1g% casein and 25 µCi of ¹²⁵I-HSA was instilled into the peritoneal cavity (t = 0 hours). Hourly samples of peritoneal dialysis fluid and blood were taken for a period of 6 hours. Hourly lymph collections were made from the indwelling catheters. At the end of each experiment (t = 6 hours), the sheep were sacrificed and whatever remaining fluid in the peritoneal cavity was allowed to drain by gravity via the peritoneal catheter into a graduated cylinder. Because there would be some unknown residual volume of fluid remaining in the peritoneal cavity, an additional 1,000 ml of saline was instilled. The cavity was then opened and the contents massaged thoroughly to ensure that any residual ¹²⁵I-albumin solution was mixed well with the added saline. Three samples were collected with massaging of peritoneal cavity contents between each sample.

To determine the concentration of

radioactivity (in cpm/ml), two 1.0 ml aliquots of each of the lymph, blood plasma, infusate and peritoneal lavage samples were placed in a multi-channel gamma spectrometer (1282 CompuGamma CS, LKB Wallac-Pharmacia, Turku, Finland) with appropriate window settings and background subtraction. To ensure that the measured ¹²⁵I-HSA in any sample was protein-associated, a second set of aliquots was assayed after precipitation with 10% trichloroacetic acid. The total amount of radioactivity in any one sample was calculated by multiplying the concentration of radioactivity in that sample by the volume of that sample.

Estimation of Peritoneal Volumes

To estimate the volume in the peritoneal cavity at t = 6 hours after much of the fluid was drained into a graduated cylinder, a dilution of radioactivity method was used as described in an earlier publication (18). To calculate the intraperitoneal volume (IPV_t) at each time point, one must take the loss of tracer from the peritoneal cavity into account (19-21). IPV_t can be calculated from the difference in the amount of tracer infused and the amount of tracer lost from the peritoneal cavity divided by the tracer concentration or:

$$IPV_t(ml) = \frac{VoCo - \{(TDR \cdot \Delta t \cdot C_{geo}) + VsC\}}{C_t} \quad (1)$$

where VoCo is the volume and concentration of tracer instilled at time 0, TDR is the fluid loss rate based on tracer disappearance, C_{geo} is the geometric mean of the initial (C₀) and final (C₆) tracer concentration in the peritoneal cavity, VsC is the volume taken from the peritoneal cavity for sampling purposes and C is the concentration of radioactivity in this sample. To calculate the IPV₁ at 1 hour

$$IPV_1(ml) = \frac{VoCo - \{(TDR \cdot 1 \cdot C_{geo}) + VsC_{1min}\}}{C_1} \quad (2)$$

where $C_{1\min}$ is the tracer concentration at 1 min, and C_1 is the intraperitoneal tracer concentration at 1 hour. To calculate the intraperitoneal volume at 2 hours

$$IPV_2(ml) = \frac{V_1 C_1 - \{TDR \cdot 1 \cdot C_{geo}\} + V_s C_1}{C_2} \quad (3)$$

where $V_1 C_1$ is the peritoneal volume and tracer concentration at 1 hour and C_2 is the intraperitoneal tracer concentration at 2 hours and so on. TDR is the fluid loss estimated from tracer disappearance (TD) from the peritoneal cavity.

$$TD (ml) = (V_0 C_0 - V_6 C_6) / C_{geo} \quad (4)$$

where V_0 and C_0 represent the volume of fluid instilled (ml) and the concentration of radioactivity (cpm/ml) in the peritoneal cavity at $t=0$ hours, V_6 and C_6 are the volumes and concentrations of tracer in the peritoneal cavity at the end of the experiment (6 hours). The fluid loss rate based on tracer disappearance is equivalent to TD / t_f .

Analysis of the Data

The data was assessed with analysis of variance. We interpreted $P < 0.05$ as significant.

RESULTS

Development of Peritonitis Model

Experiments that utilize live bacteria are difficult to interpret because the microorganisms grow at variable rates and the host inflammatory and immune responses add further variation that cannot be controlled. The induction of peritonitis was attempted with glutaraldehyde-killed *S. aureus* but the injection of up to 10^9 bacteria failed to elicit significant numbers of leukocytes into the

cavity over a 6 hour period. Subsequent attempts focused on a sterile peritonitis model using casein based on the method described by Gans et al (22).

In preliminary studies in which 6g% casein was added to the Dianeal infusate, the peritoneal neutrophil concentrations increased with a peak at 12 hours following infusion (data not illustrated). By 5 days, few neutrophils could be recovered. The concentration of mononuclear cells (predominantly macrophages) increased slowly. Over the 5 day duration of the experiments, the concentration of macrophages was highest on the 5th day. Lymphocyte concentrations in the peritoneal fluid were very low and changed little over the experimental period. Since some animals appeared to react adversely to 6g% casein, we experimented with different concentrations of casein. We observed that 1g% casein induced similar cell profiles in the peritoneal cavity but did not appear to cause any animal discomfort. For these reasons, all subsequent quantitative experiments were performed with 1g% casein.

Some consideration was given to the time at which the casein was administered into the peritoneal cavity relative to the measurement of lymph parameters. One might administer the casein solution a few days prior to the experiment (with predominantly a mononuclear cell infiltrate) or at the time of the experiment (which produces a PMN infiltrate). In the experiments reported here, the casein was added to the Dianeal® 4.25% solution just before the infusion. We monitored peritoneal and lymphatic volumes and cell content over a 6 hour period.

Peritoneal Volume Changes

Fig. 1 illustrates the volume in the peritoneal cavity. With the mean weight of the animals at 31.2 kg, the mean instilled volume was 1572 ± 51 ml. Following infusion, peritoneal volumes increased to a peak of 2119 ± 77 ml at 3 hours and declined to 1964 ± 68 ml at the 6 hour point.

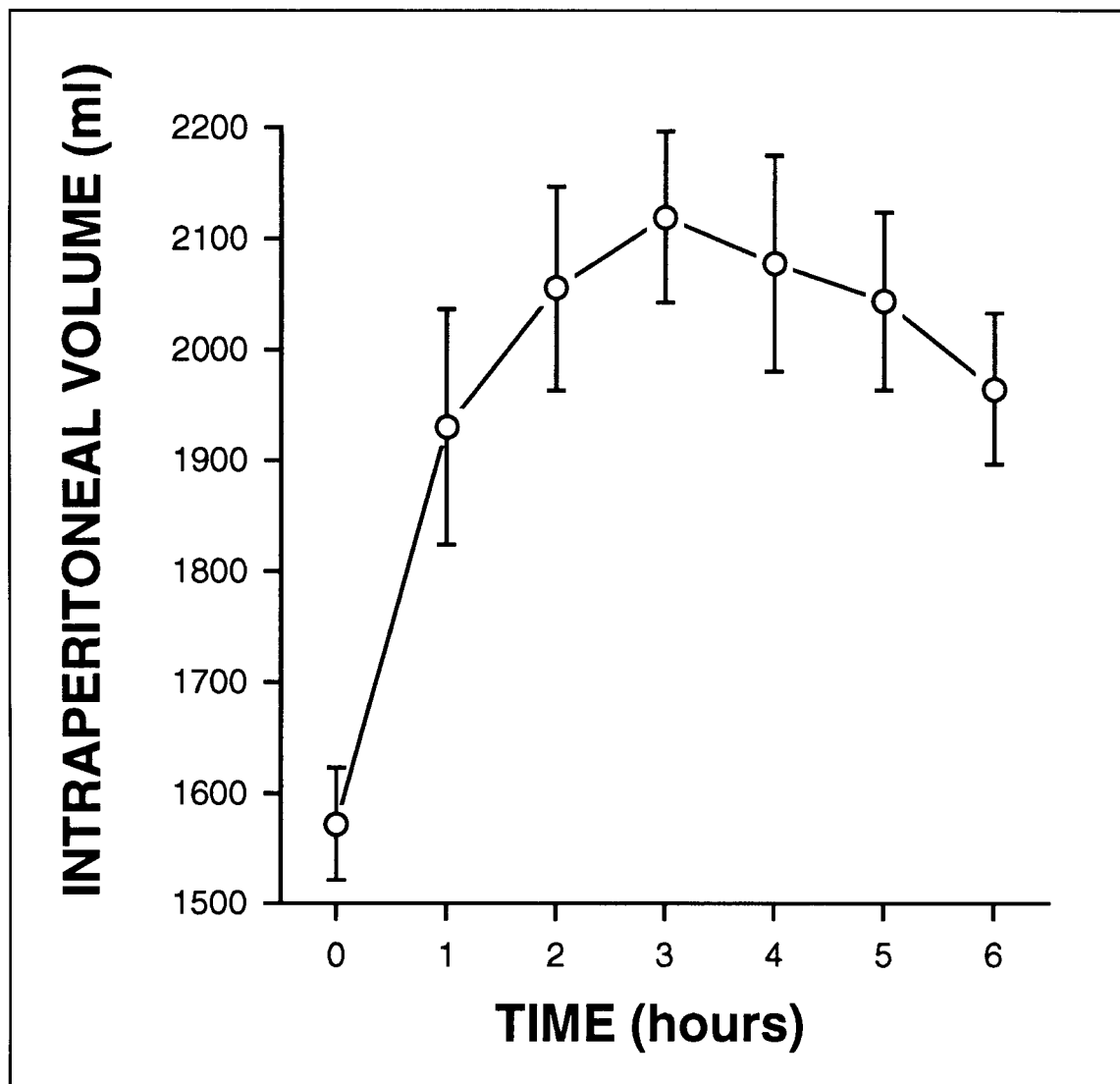


Fig. 1. Intra-peritoneal volume following infusion of 50 ml/kg volumes of Dianal® 4.25% + 1g % casein. Volumes were calculated as described in Materials and Methods.

Cell Counts in the Peritoneal Cavity

Fig. 2 illustrates the total number of cells recovered in the peritoneal cavity at each time point. The number of macrophages and lymphocytes remained relatively constant but the total number of neutrophils increased steadily to reach approximately 9×10^9 cells by 6 hours.

Lymph Flow Changes in the Caudal, Thoracic and Prescapular Lymph Compartments

The efferent lymphatic duct from the caudal mediastinal lymph node collects lymph from the diaphragm and is a major route (along with the parasternal pathway leading to the right lymph duct) by which liquid and solutes are removed from the peritoneal cavity.

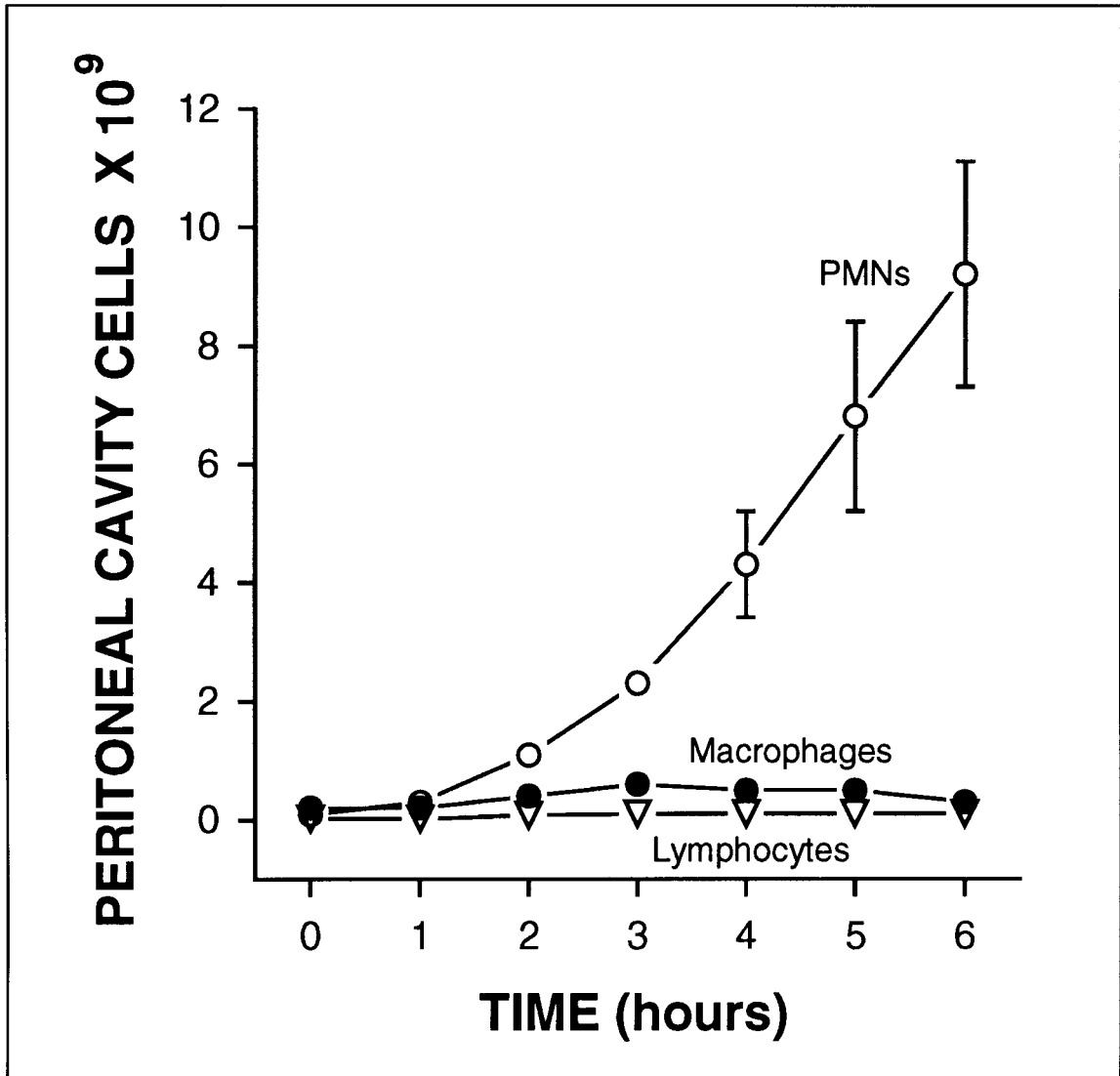


Fig. 2. Appearance of cells in the peritoneal cavity following the infusion of 50 ml/kg volumes of Dianeal® 4.25% + 1g % casein. The total number of PMNs (open circles), macrophages (closed circles) and lymphocytes (open triangles) were calculated from the volumes and the concentration of cells at each time point. The increase in peritoneal PMNs was significant.

Caudal flow rates averaged 4.3 ml/hr before infusion and increased up to a maximum of approximately 25 ml/hr after the Dianeal-casein infusion (Fig. 3). The thoracic duct also contributes to peritoneal lymph drainage. We noted in this and in previous studies (16) that thoracic duct flows tend to decline during the 3 hour control period leading up to the

infusion of dialysis solution into the cavity. This may be due to the restriction on flow caused by the indwelling catheter which is at least partially relieved when the catheter is cut to permit collection of lymph for the experiment. In any event, thoracic duct flow rates appeared to decline from approximately 79 ml/hr to between 30 and 40 ml/hr between 3

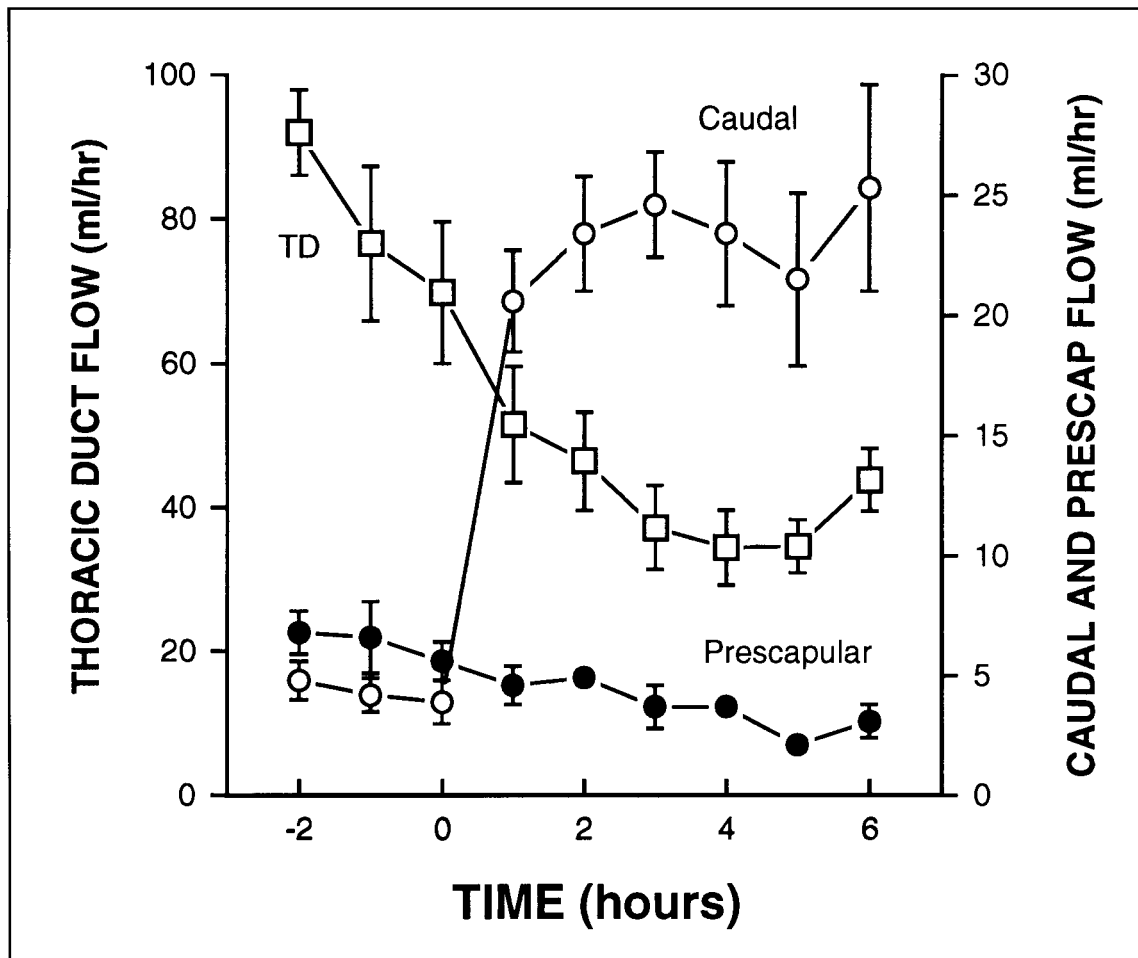


Fig. 3. Lymph flow from the caudal mediastinal vessel, the thoracic duct and prescapular lymphatic following intraperitoneal infusion of 50 ml/kg volumes of Dianeal® 4.25% + 1g % casein. Open circles - caudal flows, closed circles - prescapular flows, open squares - thoracic duct flows. The Dianeal-casein solution was infused at 0 time. All points are mean \pm SEM, n = 5. A single factor ANOVA over time with comparisons back to baseline revealed significant increases in the caudal lymph flow rates and decreases in thoracic duct flows (compared to baseline, thoracic duct flows between 2 and 6 hours were significantly depressed). Prescapular flows tended to decline but only the flows at 5 and 6 hours were significantly depressed relative to baseline.

and 6 hours after infusion. The flow rate from the prescapular lymphatic (which does not drain the peritoneal space) also declined from approximately 6 ml/hr to 3 ml/hr at 6 hours.

Cell Profiles in the Lymph Compartments

The concentration of total cells in each of the 3 lymph compartments declined after infusion of the Dianeal-casein solution (Fig. 4).

Figs. 5, 6 and 7 illustrate the total cell outputs (product of the volume and cell concentration) in the caudal, thoracic and prescapular lymph compartments respectively. In all lymph compartments, lymphocyte outputs declined. The magnitude of this reduction was 78% for caudal, 90% for thoracic duct and 83% for prescapular lymph compartments. In the caudal lymphatic, the total number of neutrophils in the lymph increased from 3.1 x

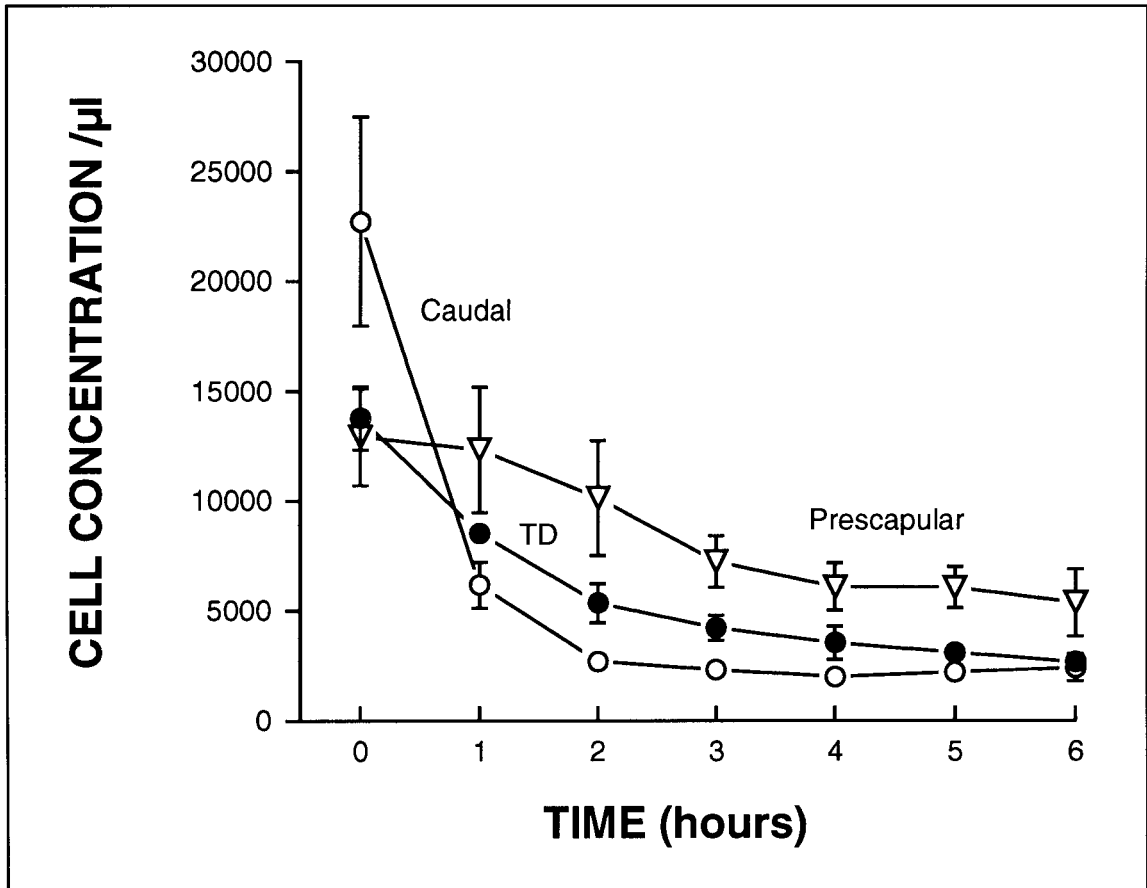


Fig. 4. Total cell concentrations in each of the lymph compartments. Caudal lymph (open circles), thoracic duct lymph (closed circles) and prescapular lymph (open triangles). The decline in cell concentration in the caudal and thoracic duct lymph was significant. In prescapular lymph, there was a significant time effect but only the cell concentrations at 6 hours were significantly different than baseline.

10^6 in the control period to 4.6×10^7 at 6 hours (Fig. 5). There was no evidence of increased entry of neutrophils into the thoracic duct or prescapular vessels over the duration of the experiment (Figs. 6 and 7).

DISCUSSION

Lymph is drained from the peritoneal cavity in sheep via three anatomically distinct pathways (14). In the first, liquid and solutes are removed from the peritoneal cavity by diaphragmatic lymphatics which pass into the caudal sternal lymph nodes. Efferent

lymphatics from these nodes transport the material to the cranial sternal lymph nodes. Efferent cranial sternal lymphatics then convey the material either directly or indirectly, via tracheal lymphatic trunks, to the right lymph duct. Unfortunately, these lymphatics are very difficult to cannulate. In the second pathway, material is transported from the peritoneal cavity by diaphragmatic lymphatics which pass into the caudal mediastinal lymph node. Efferent lymphatic ducts from the caudal mediastinal lymph node then transport the material to the thoracic duct. Under normal conditions, a significant

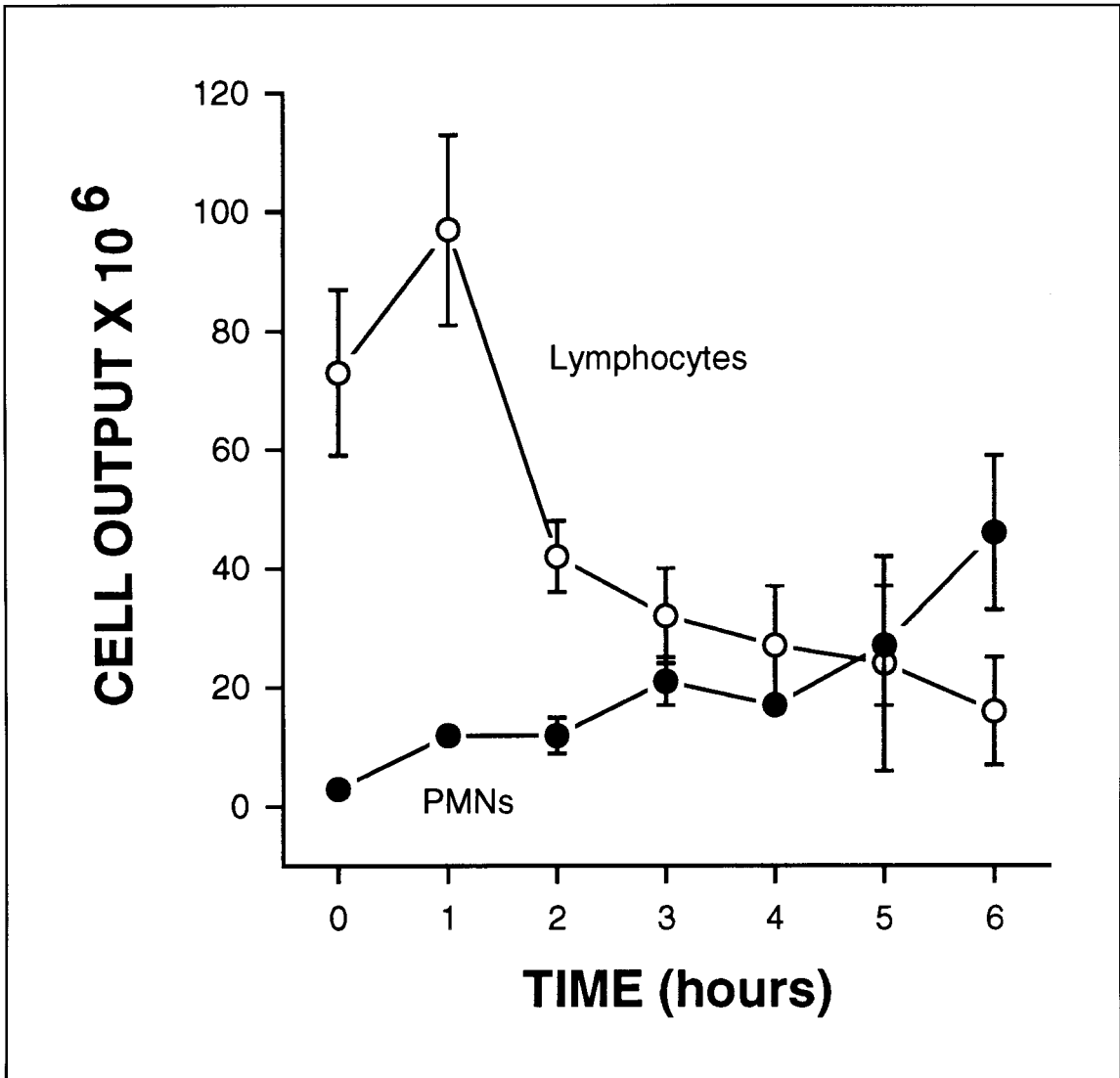


Fig. 5. Cell outputs (volume X cell concentration) in caudal lymph. Lymphocytes (open circles), PMNs (closed circles). The reductions in lymphocyte output and the increases in PMN outputs in caudal lymph were significant.

portion of caudal lymph flow comes from the lung (23). However, with an artificial ascites, the major contribution to flow comes from the peritoneal cavity (15,16,18,24). It has generally been assumed that the thoracic duct does not play an important role in peritoneal lymph drainage in certain species (25). Under some circumstances however, the thoracic duct drainage of the peritoneal cavity may be very important. In humans, thoracic duct

lymph flow increases dramatically in hepatic cirrhosis. Flow may increase from 1 to 2 liters/day to 8 to 10 liters/day with the development of ascites (26). In the sheep, it is clear that liquid and solutes cross the mesothelial lining of the abdominal viscera and are removed from the interstitium by afferent visceral lymphatics. Material taken up in this manner is ultimately transported to the thoracic duct by efferent visceral lymphatics.

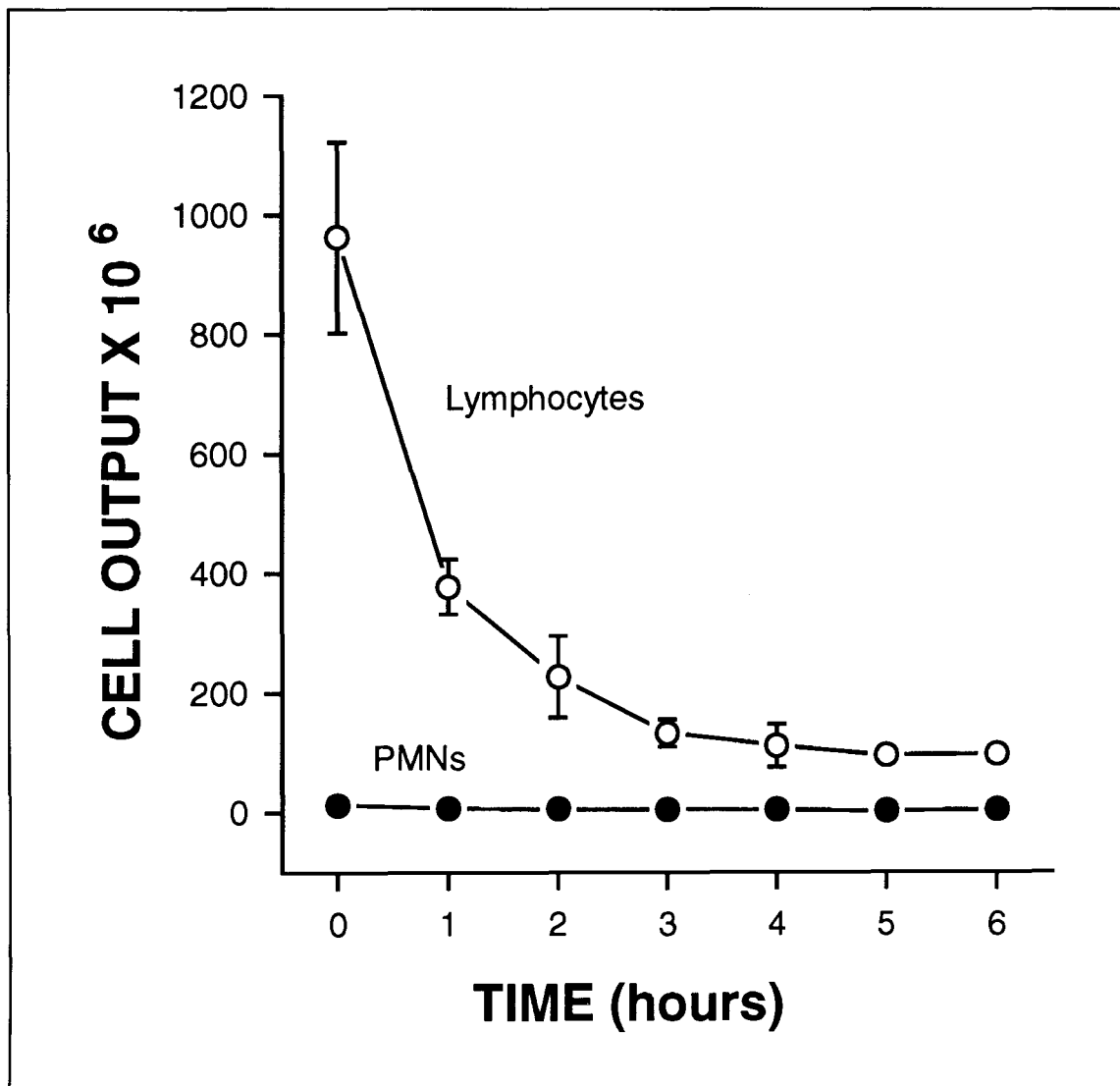


Fig. 6. Cell outputs (volume \times cell concentration) in thoracic duct lymph. Lymphocytes (open circles), PMNs (closed circles). The reduction in lymphocyte output in thoracic duct lymph was significant. There were no significant changes in PMN outputs.

Both caudal and thoracic duct pathways are accessible to cannulation. Using ¹²⁵I-HSA as a lymph flow marker and combining data from non-cannulated and cannulated sheep, we previously estimated peritoneal lymph drainage with an artificial ascites. Following infusion of 50 ml/kg volumes of Ringer's solution, peritoneal lymph drainage was estimated to be 1.35 ml/hr/kg with 66% of this

flow drained by the caudal vessel, 22% by the parasternal pathway (right lymph duct) and 12% by the thoracic duct (18).

The addition of 1g% casein to the dialysate, induced cellular changes in the peritoneal cavity that were consistent with an acute inflammatory response. Large numbers of PMNs were elicited into the cavity over 6 hours similar to that observed in patients

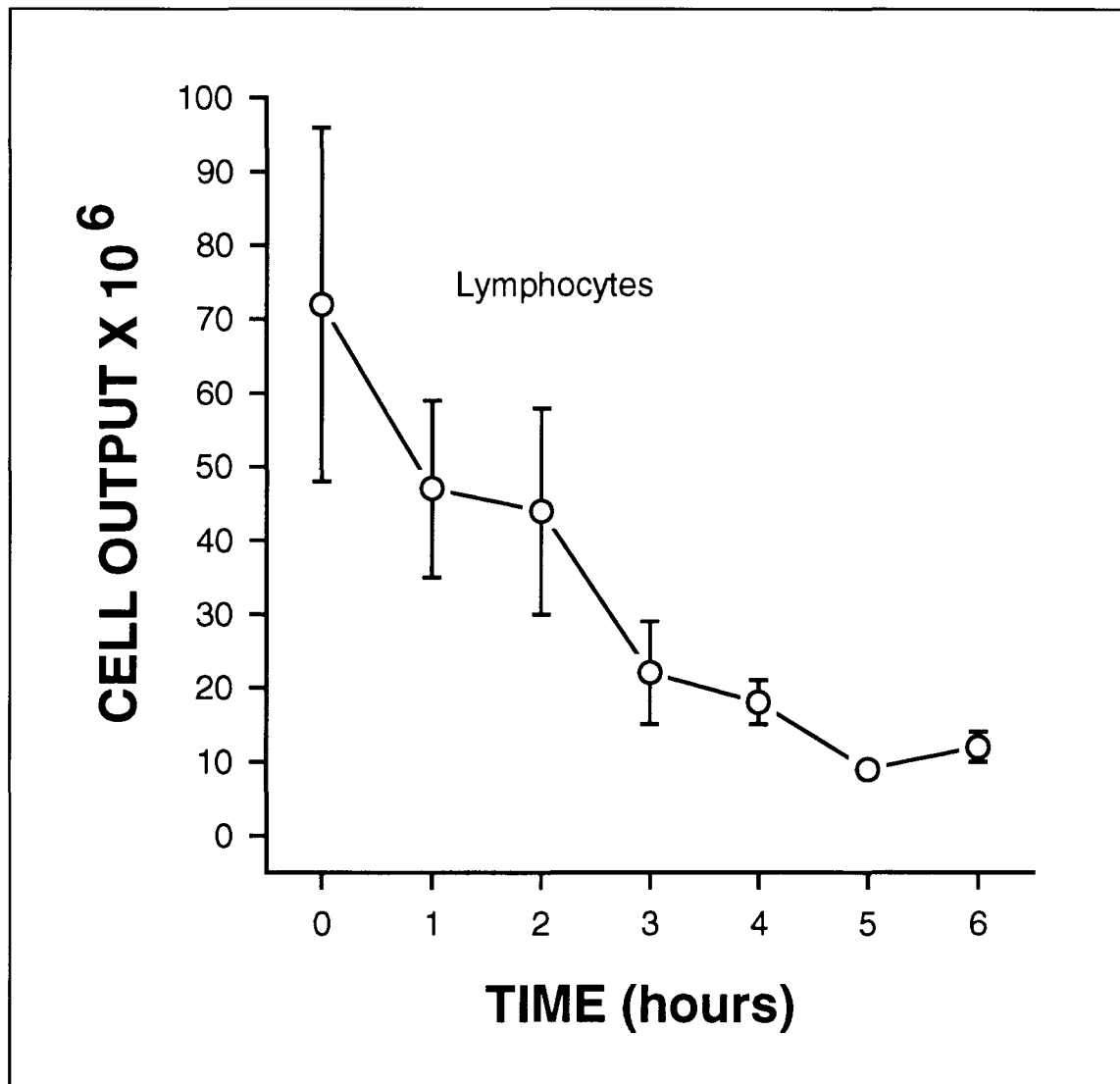


Fig. 7. Cell outputs (volume X cell concentration) in prescapular lymph. Lymphocytes (open circles). Analysis of the decrease in lymphocyte cell outputs in prescapular lymph revealed a significant overall time effect. However, comparisons of individual points back to baseline did not reveal significant differences.

developing peritonitis. The expression of adhesion molecules and chemotactic cytokines by the peritoneal mesothelial cells may be important in this regard (27).

Considering all of the lymph compartments analyzed in this study, the most dramatic changes were observed in the caudal lymph. The infusion of Dianeal-casein into the peritoneal cavity resulted in an increase in

flow rates in the efferent lymphatic draining the caudal mediastinal lymph node. This is to be expected since part of the infusate would drain through the diaphragmatic stomata into the afferent lymphatics on the pleural surface of the diaphragm (14). A peritonitis-induced widening of the stomata may facilitate the transfer of fluid and inflammatory cells from the peritoneal cavity into the draining caudal

lymph (28,29). In addition, large numbers of PMNs were observed in caudal lymph. While the number of PMNs increased, the lymphocyte output declined. The decrease in the lymphocyte outputs in the caudal lymph could be partly due to the dilution of this lymph with the relatively acellular fluid entering the duct from the peritoneal cavity. In addition to this, however, antigen has the ability to depress lymphocyte output from a lymph node.

The decreased output of lymphocytes that classically occurs within the first hour of antigen challenge was described originally by Hall and Morris (30), and this has been confirmed in many other studies and with a variety of antigens (31). The immediate drop in lymphocyte numbers in the efferent lymph is due largely to the temporary detention of lymphocytes which are already within the lymph node (32). Host factors such as interferons or other cytokines are probably responsible (33) and may orchestrate the rapid induction of adhesion molecules on the lymphatic endothelial cells within the lymph sinuses of the node. Previous studies have demonstrated that, when the efferent lymph is devoid of lymphocytes, there is in fact an increase in the entry of lymphocytes from the blood into the lymph node and this may be a reflection of the antigen-induced increase in blood flow to the lymph node (34). This recruitment is subsequently seen as an increased output of lymphocytes in the lymph, usually 24-48 hours after the antigenic challenge (35). If we were to follow the present experiments for longer than 6 hours, we predict that the lymphocyte output in the caudal lymph would increase significantly.

Previous studies have demonstrated that some intraperitoneally-injected tracer was recovered in the thoracic duct (14-18,36). However, we did not observe an increase in PMNs in the thoracic duct over 6 hours. Perhaps a small proportion entered the mesenteric nodes but had insufficient time to reach the efferent (thoracic duct) lymph. Alternatively, once the PMNs had actively migrated into the peritoneal cavity, there would not

appear to be a stimulus that would entice them to actively migrate back into the visceral tissues. Once in the peritoneal cavity, it seems likely that they would be carried passively through the relatively low resistance pathways leading to the diaphragmatic vessels and ultimately into caudal lymph. It seems less probable that the PMNs would be carried passively through the many barriers provided by the mesothelium and the tissues of the visceral organs to reach afferent visceral lymphatics.

It is common for the flow rate of the regional lymph to increase during an immune or inflammatory response (37). As noted earlier, ascites in humans secondary to hepatic cirrhosis results in large increases in thoracic duct lymph flow (26). However, in the studies reported here, thoracic duct flows declined although this data was difficult to interpret since flows were also declining in the control period. This control flow pattern is consistently observed with this preparation and may be due to the resistance offered by the thoracic duct catheter. We cannulated the thoracic duct 24 hours before the experiment. The catheter was looped and the outflow end inserted into the duct at a downstream location. In this way, thoracic duct lymph continued to flow to the venous circulation as the sheep recovered from the anesthetic. In the morning of the experiment, the catheter was cut and the lymph collected. Some 'backup' of lymph may have occurred due to catheter resistance with flows steadily decreasing until a new equilibrium was reached. We have considerable experience with this preparation and have observed that flows become stable after the 3 hr control period (16).

After the Dianeal®-casein solution was infused, thoracic duct flow rates declined further. In addition, prescapular flows also declined. These flow patterns may have been due to diminished blood volume as a result of the dialysis and exudative losses into the peritoneal cavity. In addition, one might argue that the hypertonic dialysate may remove fluid not only from the vasculature, but also from the lymphatics in the visceral organs. In

previous studies we noted that the infusion of similar volumes of Dianeal® 4.25% (measured osmolality 486 ± 1.09 mOsm/L, $n=9$) resulted in a reduction of prescapular but not thoracic duct lymph flow (16). The osmolality of the Dianeal® -casein mixtures was very similar (measured osmolality of Dianeal® 4.25%-1% casein solution = 491 ± 1.73 mOsm/L, $n=8$). The decrease in vascular volume may reduce filtration and could account for the reduction in prescapular flow rates. While the infusion of Dianeal® alone did not reduce thoracic duct flow rates, it is possible that blood volume reduction was greater in the sheep receiving Dianeal® -casein solution due to the extra loss of fluid caused by an inflammatory exudate.

Another issue to consider is that lymph flow is the result of factors that regulate the entry of liquid and protein into the initial lymphatic ducts and factors that regulate the propulsion of lymph along the lymphatic network. These latter forces may include active as well as passive factors. Many lymphatics have the capacity to contract and in concert with one-way valves, lymph is pushed from the interstitium to the bloodstream. This intrinsic lymph pump can be modulated by neurogenic mechanisms as well as by humoral factors (38). It is possible that the major cellular and physiological changes associated with peritonitis may alter the pumping activity of the visceral lymphatic vessels. If true, this may indicate an as yet undefined link between antigens or host-derived products of interactions with antigens and lymph pump regulation.

Lymphocyte outputs declined in both the thoracic duct and prescapular vessels. One might argue that this is due to the reduced lymph flows but it is also evident that the concentration of lymphocytes declined in both of these lymph compartments (*Fig. 4*). Clearly, the combination of decreased flows and cell concentrations contributed to the depression of lymphocyte outputs. Under normal physiological conditions, the lymphocyte output of a lymph node is relatively constant and small decreases in flow are accompanied

by corresponding increases in lymphocyte concentration (25). In the present studies there appeared to be systemic effects caused by the casein antigen since there is no evidence that the prescapular lymph node drains the peritoneal cavity (14). Presumably cytokines or other factors reach the prescapular lymph node from the blood. These factors could have entered the vasculature from the peritoneal cavity via the right lymph duct which was not cannulated or possibly, by entering the capillary circulation directly from the peritoneal cavity.

A number of important issues need to be investigated further. One question relates to total lymph drainage of the peritoneal cavity. Is lymph drainage increased or decreased in casein-induced peritonitis relative to infusions of Dianeal alone? The flows reported here are from individual lymphatic catheters and represent lymph flow from the total drainage area of the particular duct in question. In the caudal lymphatic, it seems clear that the increase in flow rates is due to the transfer of liquid from the peritoneal cavity into this lymphatic compartment. However, changes in thoracic duct flows may be misleading. This central lymphatic drains many organs and tissues in addition to the peritoneal space. A more detailed assessment of peritoneal to thoracic duct transport of fluid is required using tracer molecules and mass balance analysis. By measuring the mass of protein in a lymph compartment relative to the concentration of tracer in the peritoneal cavity, one can estimate the contribution of peritoneal lymph drainage to flow rates (15-18,36). Alternatively, one can take a similar approach in non-cannulated animals by monitoring the mass of intraperitoneally administered tracer in the bloodstream to estimate total lymphatic drainage of the peritoneal cavity (19,36). These studies are currently in progress.

It seems possible that the relationship between lymph drainage and peritonitis may change depending on where one monitors lymphatic parameters along the continuum of

changes associated with progression from acute to chronic inflammation. The quantitative experiments reported here were performed over 6 hours. Since the casein was added to the peritoneal cavity at time = 0, the peritonitis had only 6 hours to develop and as a consequence, assumed the characteristics of an acute inflammatory reaction. However, the cellular profiles in the peritoneal cavity would have been quite different if the casein had been infused some time before the experiments were performed. An assessment of lymph drainage of the peritoneal cavity in chronic inflammation may yield different results. Chronic peritonitis may lead to scarring of the peritoneal surface of the diaphragm which may reduce absorption of macromolecules from the cavity (39).

In summary, the most notable changes in lymph flow and inflammatory cell content in a sterile peritonitis model in sheep occurred in lymphatics that drain the peritoneal space through the diaphragm. The lymph drainage into the visceral lymphatics leading to the thoracic duct decreased and few inflammatory cells entered this lymph. Since lymphocyte cell outputs declined in all lymphatics monitored in this study including those that did not drain the peritoneal cavity, we concluded that intraperitoneal casein had some systemic antigenic effect.

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