Studies using animal models of peritoneal dialysis (PD) have commonly induced acute peritonitis by intraperitoneal (IP) administration of lipopolysaccharide (LPS). We compared the effects of peritonitis induced by IP administration of either LPS or zymosan on inflammatory parameters [dialysate leukocyte counts and dialysate concentrations of prostaglandin E2 (PGE2) and vascular endothelial growth factor (VEGF)] and peritoneal transport of fluid, small solutes (glucose), and macromolecules (total protein) in a mouse model of PD. Eighteen hours after induction of peritonitis, mice were studied by injecting 2 mL of 4.25% dextrose-containing PD solution into the peritoneal cavity for a 2-hour dwell. Concentrations of glucose, total protein, PGE2, and VEGF were determined in the dialysate effluent. Acute peritonitis induced by IP administration of LPS induced changes in peritoneal transport similar to those observed during clinical PD, but without a significant increase in the dialysate leukocyte count. In contrast, acute peritonitis induced by IP administration of zymosan induced a large increase in dialysate leukocyte count, more substantial changes in peritoneal transport, and increases in dialysate PGE2 and VEGF concentrations. We conclude that acute peritonitis induced by IP administration of zymosan in the mouse may be a more relevant model for clinical PD, because it produces substantial changes in peritoneal transport and leukocyte migration into the peritoneal cavity.

Key words
Inflammation, leukocytes, permeability, peritonitis, protein loss

Introduction
The development of animal models with direct relevance to peritoneal dialysis (PD) patients is a major goal of recent efforts in PD research. The rat model of acute and chronic peritoneal membrane exposure to PD solution has recently been suggested to be most relevant (1); however, mouse models are used with increasing frequency, because mice deficient in specific proteins can be a powerful scientific tool (2–5).

Although animal models of PD—and especially models of chronic exposure of the peritoneal membrane to PD solution—have been well characterized, animal models for the study of acute or chronic peritonitis are less well defined. A review of recent work reveals that acute peritonitis has been induced by intraperitoneal (IP) administration of bacteria in the rabbit (6,7), by nonsterile implantation of a peritoneal catheter in the rat and mouse (2,8), and by IP administration of lipopolysaccharide (LPS) in the rat and mouse (9–13). The latter approach is advantageous because LPS is commercially available and chemically pure, allowing for consistent and reproducible administration of the inflammatory stimulus. Only a single study has been performed to characterize alterations in peritoneal transport after IP administration of LPS to induce acute peritonitis in a mouse model (12).

In the present study, we compared acute peritonitis induced by either LPS or zymosan in our recently developed mouse model of PD (14). We hypothesized that different inflammatory stimuli would induce different degrees of inflammatory response and changes in peritoneal transport.

Materials and methods

Experimental methods
Acute experiments were performed on male C57BL/6 mice (The Jackson Laboratory, Bar Harbor, ME, U.S.A.) with initial body weights averaging 20.9 g (range: 19.0 – 26.7 g). Peritonitis was induced by IP injection (using a 27-gauge needle) of either 200 µg LPS (from E. coli 0111:B4: Sigma

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Chemical, St. Louis, MO, U.S.A.; \( n = 9 \) or 1 mg zymosan (Sigma Chemical, \( n = 9 \)) in 0.5 mL sterile saline 18 hours before an experimental dwell with PD solution. In non peritonitis control mice, no initial IP injection was given. The mice were returned to their cages until the experiment the following day.

On the day of the experiment, each mouse was first anesthetized by intramuscular injection of 100 \( \mu \text{g/g} \) ketamine and 10 \( \mu \text{g/g} \) xylazine, followed by additional doses as required to maintain anaesthesia. An experimental dwell was then performed by IP injection (using a 27-gauge needle) of 2 mL commercial PD solution containing dextrose as the osmotic agent at a concentration of 4.25\% (Delflex: Fresenius Medical Care North America, Ogden, UT, U.S.A.). After the needle was removed, a small quantity of cyanoacrylate cement (Superglue: Superglue Corporation, Rancho Cucamonga, CA, U.S.A.) was spread across the exit site to prevent fluid leakage from the peritoneal cavity. This solution was allowed to dwell for 2 hours.

At the end of the dwell period, the mouse was humanely killed, and a blood sample of 0.5 – 1.0 mL was obtained by either cardiac puncture or exsanguination after decapitation. A 1 – 2 cm midline incision was then made to surgically open the abdominal cavity. The mouse was then inverted and the fluid within the peritoneal cavity was drained as completely as possible. The drained fluid was weighed using a digital scale (XE series, Model 400: Denver Instruments, Denver, CO, U.S.A.). The fluid volume remaining in the peritoneal cavity after drainage was measured by swabbing all peritoneal tissues with sterile gauze and by determining the difference in the weight of the gauze before and after swabbing. All fluid weights were then converted to volumes, assuming a solution density of 1.0 g/mL. The drain volume of fluid in the peritoneal cavity at the end of the dwell was therefore reported as the combined total of the drained and the residual volume. All experiments were repeated 9 times under each set of experimental conditions.

**Analytical assays**
The blood sample was collected in a tube containing heparin and was centrifuged to obtain plasma for further analysis. An automated analyzer (Dade–Behring, Deerfield, IL, U.S.A.) was used to measure glucose and total protein in plasma and dialysate samples. Using commercial assay kits from R&D Systems (Minneapolis, MN, U.S.A.), prostaglandin E2 (PGE2) and vascular endothelial growth factor (VEGF) were determined in dialysate. Dialysate leukocytes were counted using a hemocytometer.

**Calculations and statistics**
The disappearance rate of glucose from the peritoneal cavity during the dwell was evaluated by calculating the dialysate concentration of glucose relative to its concentration in the initially infused dialysis solution (\( \text{D/D}_0 \)). The appearance of protein in the peritoneal cavity was evaluated by calculating the dialysate-to-plasma (D/P) concentration ratio. No corrections for differences in ultrafiltration were made to the D/D\(_0\) or D/P values.

All empirical and calculated values are reported as mean \( \pm \) standard error of the mean. Differences in measured and calculated parameters for the three study groups were evaluated using single-factor analysis of variance (ANOVA). Further statistical comparisons between control and peritonitis mice were evaluated by using an unpaired Student \( t \)-test with confidence limits modified by the Bonferroni method.

**Results**
Figures 1 – 6 show the results of the experiments. The drain volume from control mice was comparable to that reported previously (14) when using hypertonic PD solutions (Figure 1). The volume drained in LPS-induced peritonitis mice was numerically lower, but not significantly different from that in control mice. However, the drain volume in zymosan-induced peritonitis mice was significantly lower than the volume drained in control mice. Net ultrafiltration in these experiments was 0.62 \( \pm \) 0.13 mL (control mice), 0.33 \( \pm \) 0.11 mL (LPS-peritonitis mice), and –0.06 \( \pm \) 0.08 mL (zymosan-peritonitis mice).

Figure 2 shows the results for leukocytes in the dialysate remaining at the end of the dwell. We observed no significant increase in the number of leukocytes in the dialysate in LPS-induced peritonitis, but a substantial increase was observed when peritonitis was induced by IP administration of zymosan.

Peritonitis, whether induced by LPS or zymosan, altered peritoneal transport of both glucose and protein (Figures 3 and 4 respectively). Peritoneal
Peritonitis in a Mouse Model of PD

**Discussion**

The results from the present experiments demonstrate that IP administration of LPS in the mouse induces peritoneal inflammation similar to that reported by Ni *et al.* (12), with a lower drain volume (not statistically significant), more substantial disappearance of glucose from the peritoneal cavity (lower...
D/D₀ glucose), and higher peritoneal permeability to protein (higher D/P protein) than are seen in control mice. Small increases in the dialysate concentrations of PGE2 and VEGF are also seen (not statistically significant), but no significant migration of leukocytes into the peritoneal cavity is observed. In contrast, when zymosan was the inflammatory stimulus, the increase in peritoneal transport over that in control mice was more substantial, and dialysate leukocytes increased by approximately a factor of 10. Moreover, substantial increases in the dialysate concentration of both PGE2 and VEGF were observed when zymosan was used as the inflammatory stimulus.

These observations suggest that induction of peritonitis by IP administration of zymosan (as compared with LPS) in the mouse may have characteristics more similar to the peritonitis observed in clinical PD. For example, clinical peritonitis is accompanied by a large increase (factor of 10 – 100) in dialysate leukocytes (15), similar to the increase observed in the current experiments when zymosan (but not LPS) was used as the inflammatory stimulus. It should be noted that this phenomenon is not the result of a too-low LPS dose, because the LPS dose used could not be increased without causing substantial lethality. A doubling of the LPS dose would cause approximately 50% lethality (unpublished observations).

Further, the current findings confirm general observations in the literature that IP administration of LPS in the mouse induces significant systemic inflammatory effects, but that inflammation induced by zymosan is confined primarily to the peritoneal cavity (16). Consistent with that general observation, Breborowicz et al. (9) noted that IP administration of LPS in the rat resulted in systemic inflammation, as evidenced by elevated plasma cytokine concentrations. Again, minimal systemic inflammation during acute peritonitis is a phenomenon that mimics peritonitis in PD patients.

Conclusions
Our findings demonstrate that IP administration of zymosan may prove advantageous in the study of acute peritonitis in the mouse. Zymosan, like LPS, is commercially available and chemically pure, meaning that its application will likely yield a highly reproducible system for study.

Acknowledgment
This article is based on work supported by the Office of Research and Development, Medical Research Service, Department of Veterans Affairs.

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