



Contribution of Uremia to *Ureaplasma*-Induced Hyperammonemia

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ABSTRACT Lung transplant recipients (LTRs) are vulnerable to hyperammonemia syndrome (HS) in the early postoperative period, a condition typically unresponsive to non-antibiotic interventions. HS in LTRs is strongly correlated with *Ureaplasma* infection of the respiratory tract, although it is not well understood what makes LTRs preferentially susceptible to HS compared to other immunocompromised hosts. *Ureaplasma* species harbor highly active ureases, and postoperative LTRs commonly experience uremia. We hypothesized that uremia could be a potentiating comorbidity, providing increased substrate for ureaplasma ureases. Using a novel dialyzed flow system, the ammonia-producing capacities of four isolates of *Ureaplasma parvum* and six isolates of *Ureaplasma urealyticum* in media formulations relating to normal and uremic host conditions were tested. For all isolates, growth under simulated uremic conditions resulted in increased ammonia production over 24 h, despite similar endpoint bacterial quantities. Further, transcripts of *ureC* (from the ureaplasma urease gene cluster) from *U. urealyticum* IDRL-10763 and ATCC-27816 rose at similar rates under uremic and nonuremic conditions, with similar endpoint populations under the two conditions (despite markedly increased ammonia concentrations under uremic conditions), indicating that the difference in ammonia production by these isolates is due to increased urease activity, not expression. Lastly, uremic mice infected with an *Escherichia coli* strain harboring a *U. urealyticum* serovar 8 gene cluster exhibited higher blood ammonia levels compared to nonuremic mice infected with the same strain. Taken together, these data show that *U. urealyticum* and *U. parvum* produce more ammonia under uremic conditions compared to nonuremic conditions. This implies that uremia is a plausible contributing factor to *Ureaplasma*-induced HS in LTRs.

IMPORTANCE *Ureaplasma*-induced hyperammonemia syndrome is a deadly complication affecting around 4% of lung transplant recipients and, to a lesser extent, other solid organ transplant patients. Understanding the underlying mechanisms will inform patient management, potentially decreasing mortality and morbidity. Here, it is shown that uremia is a plausible contributing factor to the pathophysiology of the condition.

KEYWORDS *Ureaplasma*, hyperammonemia, lung transplantation

Thousands of lung transplants are performed every year in the United States, with numbers anticipated to grow as availability and survivability continue to improve. A primary reason for greater survivability in recent years has been the incorporation of strategies to minimize mortality caused by posttransplant infections. A contributor that has plagued lung transplant recipient (LTR) survival is hyperammonemia syndrome (HS), which occurs in around 4% of LTRs (1, 2). Ammonia (NH₃) is a neurotoxin that, when present in excess, transverse the blood-brain barrier and causes cerebral edema (3, 4).

HS following lung transplantation typically progresses from early identification of elevated blood NH₃ levels or hyperammonemia (HA), causing altered mental status resulting in confusion, lethargy, obtundation, and agitation, to eventual cerebral

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edema, resulting in seizure, coma, and often death (5–9). HS that presents in LTRs is atypical in that these patients do not have underlying liver disease or urea cycle disorders. Further, nontargeted interventional efforts to suppress endogenous NH_3 production biochemically or physiologically and/or increase NH_3 excretion have had minimal impact.

Recently, *Ureaplasma urealyticum* and *Ureaplasma parvum* were linked with HS in LTRs (10); the airways of every LTR presenting with unexplained HS studied ($n = 13$) tested positive for *Ureaplasma* species, likely of donor origin. Further evidence that *Ureaplasma* species are causative agents of HS in LTRs has come by way of *in vivo* studies with murine models, where it was shown that intratracheal combined with intraperitoneal infection with either *U. urealyticum* or *U. parvum* resulted in HA (11, 12). *Ureaplasma* species, which are normally considered commensal microbiota of the urogenital tract, produce a potent urease that splits urea into NH_3 and CO_2 as a means of ATP synthesis, powered by the NH_3 gradient generated across the membrane (13, 14). Interestingly, 95% of ATP generated by *Ureaplasma* species is urea dependent, making it a requirement for growth (15). The high level of NH_3 production from LTR *Ureaplasma* infection can exceed the capacity for detoxification by the host.

While unexplained HS has been described in non-LTR transplant patient populations (2, 16–30), the prevalence rate of ~4% seems highest, by far, in the LTR population. This suggests that the lung transplant scenario is specifically well suited to microbe-driven HS. LTRs are also particularly vulnerable to posttransplant uremia (31, 32), potentially providing an abundance of substrate for ureaplasma ureases, leading to NH_3 overproduction, overwhelming the detoxification capacity of the host.

Here, the possibility that elevated blood urea in LTRs could potentially contribute to production of pathological levels of NH_3 by *Ureaplasma* species was investigated. We hypothesized that, compared to nonuremic conditions, uremic conditions would result in increased NH_3 production by ureaplasma ureases both *in vitro* and *in vivo*, ultimately leading to a greater incidence of HS.

RESULTS

All *Ureaplasma* isolates produced significantly more ammonia under conditions related to uremia. For all *U. parvum* and *U. urealyticum* isolates tested, growth in media containing 50 mg/dL urea (uremic conditions) resulted in significantly greater NH_3 production than in media containing 10 mg/dL urea (normal conditions) over 24 h (Fig. 1). Isolates grown under uremic conditions produced, on average, 1777 (standard deviation [SD] = 264) $\mu\text{mol/L}$ more NH_3 than those grown under normal conditions. NH_3

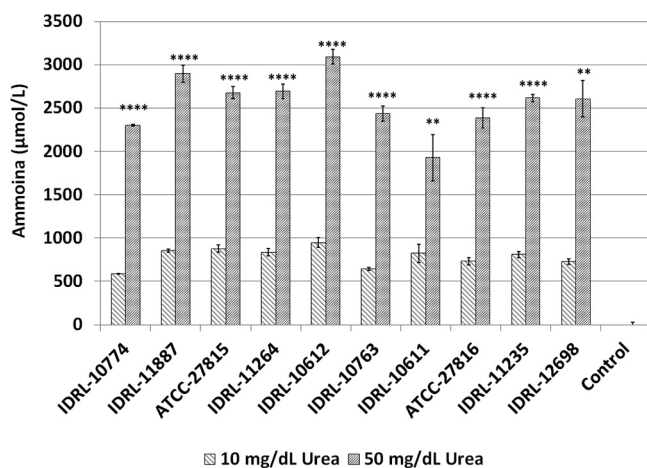


FIG 1 Average ammonia production by *Ureaplasma* isolates over 24 h under normal and uremic conditions. Isolates of *U. parvum* or *U. urealyticum* were grown in the dialyzed flow system under normal (10 mg/dL urea) or uremic (50 mg/dL urea) conditions for 24 h. The experiments were performed in triplicate. The control was uninfected medium. The significance between conditions for each isolate was determined via two-tailed unpaired *t* tests. **, $P \leq 0.01$; ****, $P \leq 0.0001$.

production was not significantly different between species, with *U. parvum* and *U. urealyticum* isolates producing averages of 1,854 $\mu\text{mol/L}$ (SD = 67) $\mu\text{mol/L}$ and 1,731 $\mu\text{mol/L}$ (SD = 131) more NH_3 , respectively, under uremic conditions. There was no noticeable difference between patient respiratory isolates and commercially available urogenital isolates. The average difference in NH_3 production between uremic and normal conditions for all patient respiratory isolates was 1,794 $\mu\text{mol/L}$ (SD = 291) versus 1801 $\mu\text{mol/L}$ (SD = 72) and 1,655 $\mu\text{mol/L}$ (SD = 119) for *U. parvum* (ATCC 27815) and *U. urealyticum* (ATCC 27816) urogenital isolates, respectively. Between uremic and normal conditions, 24-h color changing units (CCU) counts were not significantly different (Fig. S2), indicating that NH_3 production was not a result of different numbers of bacteria.

Increased ammonia production under uremic conditions was not due to increased urease production in two isolates of *U. urealyticum*. Quantitative PCR (qPCR) was used to measure *ureC* gene copies, and quantitative reverse transcription PCR (qRT-PCR) to measure *ureC* transcripts over time for one clinical respiratory isolate (IDRL-10763) and one commercially available urogenital isolate (ATCC-27816) of *U. urealyticum*. It was found that, despite a significant increase in NH_3 production for both isolates in the 50 mg/dL urea preparation, and despite similar endpoint populations (IDRL-10763: 7.23×10^8 and 7.84×10^8 copies/mL; ATCC-27816: 9.24×10^8 and 7.84×10^8 copies/mL for 10 and 50 mg/dL urea, respectively), rises in *ureC* transcripts were similar across conditions (Fig. 2). This indicates that greater urea availability does not result in increased urease production by *U. urealyticum*.

Uremic mice infected with an *Escherichia coli* strain expressing the *U. urealyticum* gene cluster exhibited elevated blood ammonia levels compared to nonuremic mice. It was determined whether an *E. coli* strain harboring the *U. urealyticum* gene cluster (33) would produce more NH_3 in uremic compared to nonuremic mice. Mice were immunosuppressed for 7 days with methylprednisone, tacrolimus, and mycophenolate mofetil, and half of the animals were administered 40 g/L urea *ad libitum* in their drinking water beginning 10 days prior to infection, resulting in elevated blood urea nitrogen (BUN) levels for urea-fed mice in comparison to control mice. The animals were infected intratracheally (IT) and intraperitoneally (IP) with 10^7 to 10^8 cells/mL of the urease-positive strain, the wild-type control, or saline + 0.1% agar (vehicle control). After 24 h, the animals were sacrificed; their blood was collected for NH_3 measurement with a point-of-care meter (Woodley Equipment Company Ltd., WD5502 PocketChem

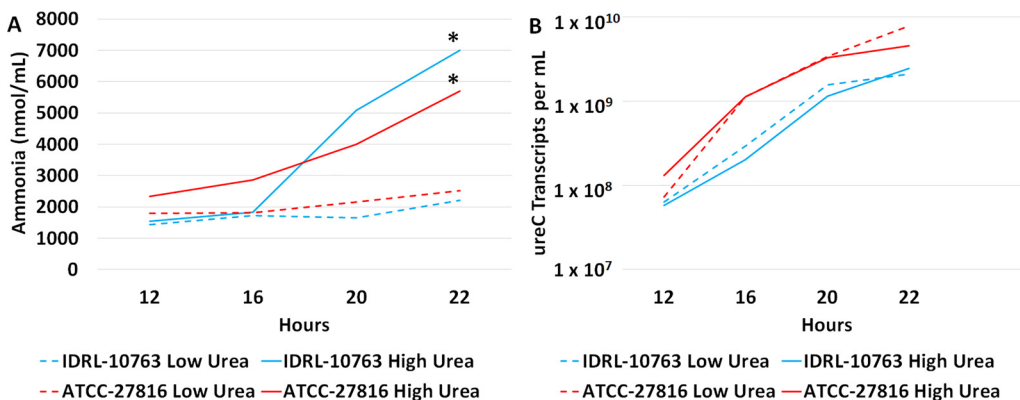


FIG 2 *ureC* transcript levels are similar with normal and elevated urea availability. 10^5 CFU/mL of *U. urealyticum* IDRL-10763 or *U. urealyticum* ATCC-27816 was grown in 10 mL 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES)-buffered U9 for 2 h at 37°C and added to a 1,000-kDa-pore-size dialysis tube that was submerged in a 250-mL flow bottle containing 100 mM MES-buffered U9 with either 10 or 50 mg/dL urea with a flow rate of 2 mL/h. (A) NH_3 was measured via the modified Berthelot reaction at each time point from the cell-free exterior of the dialysis tube. (B) Quantitative reverse transcription PCR (qRT-PCR) was performed on 50 μL of culture collected at 12, 16, 20, and 22 h and placed in DNA/RNA shield. DNA was extracted using the Maxwell rapid sample concentrator, with a standard 10^8 copies/mL extraction control from the same stock of culture in DNA/RNA shield run for each extraction group. qRT-PCR was performed on a Roche LightCycler 2.0 with crossing threshold values compared to a standard curve. The significance between urea concentrations was determined via linear regression analysis: *, $P \leq 0.05$.

BA Analyzer), blood CFU measurement (dilution and plating), and *U. urealyticum ureC* qPCR; and their lungs were harvested for total lung CFU measurement and *U. urealyticum ureC* qPCR. Despite insignificant differences between lung and blood populations (Fig. S3), infection with urease-positive *E. coli* in urea-fed mice resulted in significantly higher blood NH₃ levels than all other groups (Fig. 3).

DISCUSSION

The results of this study show that conditions representative of uremia resulted in elevated production of NH₃ by all *Ureaplasma* isolates tested. The discovery that *Ureaplasma* respiratory infections were the cause of the previously unexplained phenomenon of nonhepatic HS in early postoperative LTRs (10) has led to an improvement in patient care and a reduction in mortality rates. Still, what makes LTRs particularly vulnerable to this phenomenon remains unknown. Here, the potential impact of uremia in the early post-transplant period as a potentiating comorbidity was investigated. LTRs are frequently uremic due to acute renal failure (ARF). It has been estimated that as many as 75% of LTRs experience ARF postoperation due to renal hypoperfusion brought on by several factors, including (i) decreased circulating blood volume resulting from diuretic use to prevent pulmonary edema from leaky capillaries, (ii) nephrotoxic effects of calcineurin inhibitors, and/or (iii) reduced renal oxygenation due to postoperation hypoxia (31–36). We hypothesized that, among patients harboring a posttransplant *Ureaplasma* respiratory infection, elevated blood urea concentrations would provide greater substrate availability for ureaplasma ureases, leading to the production of NH₃ at sufficient levels to overwhelm the host detoxification capacity (Fig. 4).

Using a novel dialyzed flow system, NH₃ production of 10 isolates of *U. parvum* and *U. urealyticum* (8 clinical respiratory isolates and 2 commercially available urogenital isolates) over 24 h under conditions representative of normal (10 mg/dL) and elevated (50 mg/dL) BUN was tested. For all isolates tested, more NH₃ was produced under uremic conditions. Further, endpoint population densities were not significantly different for normal compared to uremic conditions, indicating that greater NH₃ production under uremic conditions can be credited to increased ureaplasma urease activity. Previous studies have shown that *Ureaplasma* growth capacity is highly dependent on

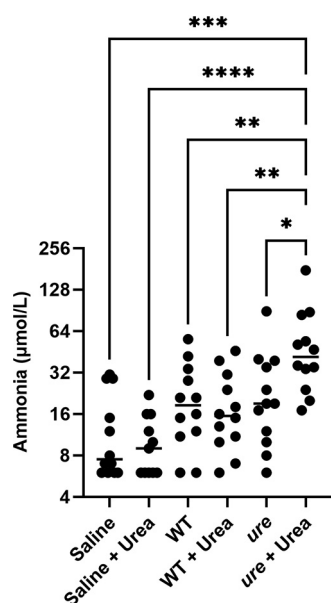


FIG 3 Blood ammonia is elevated in uremic mice infected with urease-positive *E. coli* compared to nonuremic mice infected with the same strain. Urea-fed (+ Urea) and normal mice were infected intraperitoneally and intratracheally with either wild-type (WT) or urease-positive *E. coli* (*ure*) or 0.1% saline agar. Blood NH₃ was measured 24 h later ($N = 12$ animals per group; one-way analysis of variance [ANOVA]): *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$; ****, $P \leq 0.0001$.

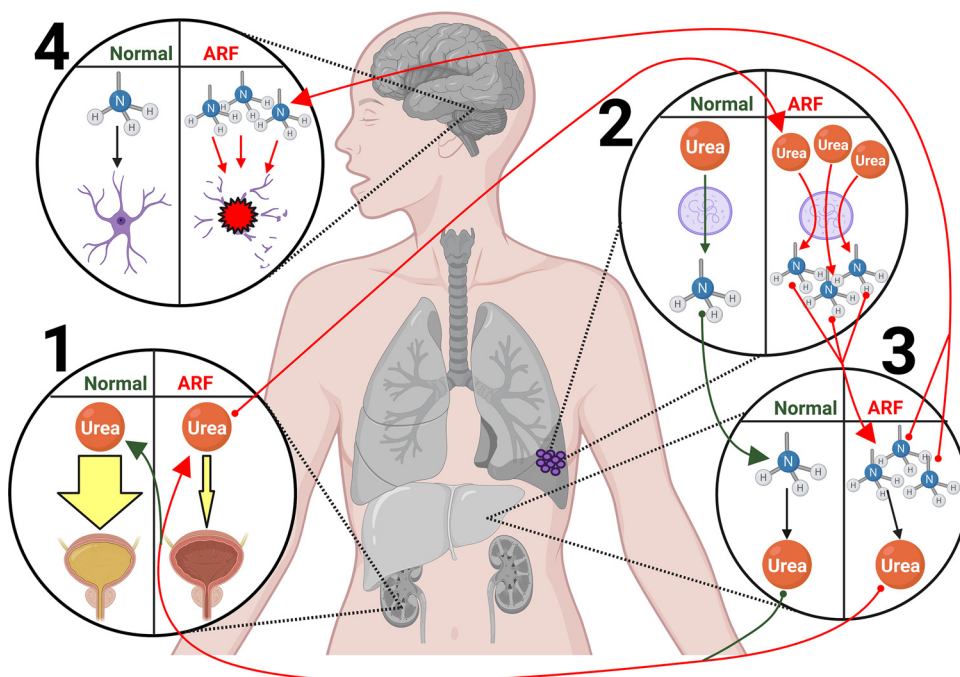


FIG 4 Hypothetical mechanism of *Ureaplasma*-induced hyperammonemia syndrome in the context of kidney dysfunction in early post-transplant lung transplant recipients. Circle 1 shows that acute renal failure (ARF) due to renal hypoperfusion results in decreased elimination of urea from the blood via urine excretion. Circle 2 shows that increased blood urea availability provides additional substrate for ureaplasma (purple cells) ureases in the infected respiratory tract, leading to greater NH_3 (blue and gray NH_3 molecule) production in comparison to normal blood urea concentrations. Circle 3 shows that elevated serum NH_3 levels overwhelm the detoxification capacity of liver urea cycle enzymes, leading to hyperammonemia. Circle 4 shows that excess serum NH_3 diffuses into astrocytes and other glial cells in the brain, causing them to swell and burst, resulting in cerebral edema and hyperammonemia syndrome. The figure was created using BioRender.

urea concentration *in vitro* (37). However, the novel flow system utilized here was designed to supply continuous urea at the desired concentration while at the same limiting increases in alkalinity to growth-inhibitory levels. With the same system, a comparison of NH_3 production and qPCR and qRT-PCR copy counts by two isolates of *U. urealyticum* showed that the increase in NH_3 production under the elevated urea condition over time was not the result of elevated urease production or greater bacterial population densities in comparison to the lower urea condition.

With this *in vitro* data in hand, an *E. coli* strain harboring the *U. urealyticum* gene cluster was used to test whether uremic mice would experience greater ureaplasma urease-induced HA compared to nonuremic mice. By supplying animals with urea in drinking water, mildly elevated BUN was achieved in comparison to mice given normal water. Uremic mice infected with the ureaplasma urease-positive strain of *E. coli* experienced elevated blood NH_3 levels in comparison to nonuremic mice infected with ureaplasma urease-positive *E. coli*, as well as all other groups, including uremic and non-uremic animals infected with either urease-negative *E. coli* or vehicle control.

There are several limitations to this study. First, these results were obtained with conditions related to mild, dietary uremia. More significant differences in BUN could be obtained with models of acute kidney injury, replicating conditions of ARF, or with non-*ad libitum* methods of urea feeding, which would theoretically lead to more significant differences in ureaplasma urease-induced HA, given the enzyme capacities demonstrated herein (Fig. 4). Second, in the *in vivo* portion of this study, only the serotype 8 urease gene cluster was investigated; it is possible that other serotypes would not have the same level of responsiveness to increased urea concentrations in the blood. To this end, further investigation will be needed to determine universality across *Ureaplasma* species. Further, *in vivo* studies were performed using an *E. coli* strain that harbors a plasmid containing the *U. urealyticum* gene

cluster and not a *Ureaplasma* isolate. This was done to provide a non-urease-producing control and because *E. coli* is easier to work with experimentally than are *Ureaplasma* species. Lastly, we chose to perform a qPCR/qRT-PCR comparison study (Fig. 2) on two representative *U. urealyticum* isolates from Fig. 1. Although the studied isolates showed increased NH₃ production under uremic conditions, it is possible that urease expression may manifest differently in certain isolates.

Taken together, these results provide strong *in vitro* and *in vivo* support for the hypothesis that posttransplant uremia may be a contributing factor to *Ureaplasma*-induced HS in LTRs. This study serves as a reminder that the effects of specific comorbidities on diseases of microbial metabolite production/overproduction should be more readily considered.

MATERIALS AND METHODS

Study isolates. The isolates of *U. parvum* and *U. urealyticum* investigated here are listed in Table 1. They include three respiratory isolates of *U. parvum* and five respiratory isolates of *U. urealyticum*, as well as one commercially available urogenital isolate of each species (from ATCC). Patient respiratory isolates are stored at the Mayo Clinic Infectious Diseases Research Laboratory (IDRL). The isolates were grown to 10⁷ CCU using a *Ureaplasma* bioreactor, as previously described (38). Aliquots in U9 media (Hardy Diagnostics) buffered with 100 mM 2-(*N*-morpholino)ethanesulfonic acid (MES) at pH 6.0, 500- μ L aliquots were frozen at -80°C until use.

An opal suppressor (under the control of an isopropylthio- β -D-galactoside [IPTG]-induced lacUV5 promoter) strain of *E. coli* harboring a plasmid containing the urease gene cluster of serotype 8 *U. urealyticum* (33) was utilized to study NH₃ production in uremic versus nonuremic mice. The plasmid carried both chloramphenicol and ampicillin resistance for selection and maintenance of urease activity. The wild-type strain lacked the plasmid and was thus urease-negative and susceptible to both antibiotics. The cultures were grown in tryptic soy broth (TSB) for 18 h, with or without antibiotics, at 37°C with shaking, after which IPTG was added, and the cultures were incubated an additional 4 h. Prior to infection, urease production by the *E. coli* strain was verified by streaking on urea agar slants (Hardy Diagnostics; R42).

Dialyzed flow system. 10-mL cultures of 10⁵ CCU/mL for all *Ureaplasma* isolates were encased in dialysis tubing (Spectra/Por Float-A-Lyzer G2 1,000-kDa dialysis device; G235073) and submerged in 250 mL of 100 mM MES-buffered U9, allowing measurement of NH₃ levels over time (Fig. 5). The entire device was incubated at 37°C, with fresh broth added and spent media removed via flow at 2 mL/hour. Urea concentrations in the growth media were varied to mimic normal and high BUN levels (10 and 50 mg/dL, respectively) in the flow chamber and inflow and were maintained at a standard deviation of 3 for the 50 mg/mL concentration and 1 for the 10 mg/mL concentration. The samples were taken from cell-free portions of the flow chamber (outside the dialysis tube) and collected into microcentrifuge tubes at 0 and 24 h, and NH₃ and urea concentrations were tested using an NH₃ assay kit (Abcam; ab102509) and a urea assay kit (Abcam; ab234052). *Ureaplasma* cells in the dialysis tubing were quantified at each collection time.

For qPCR and qRT-PCR measurements over time, 10⁵ CFU of *U. urealyticum* IDRL-10763 or ATCC-27816 was inoculated into 100 mM MES-buffered U9 medium and grown for 2 h at 37°C. After 2 h, the cultures were submerged in 250 mL of 100 mM MES-buffered U9 medium containing either 10 or 50 mg/dL urea and grown at 37°C for 22 h with 2 mL/h inflow and outflow from the exterior of the dialysis tube (i.e., cell-free portion of the system). At hours 12, 16, 20, and 22, 150 μ L of culture material was collected from the dialysis tube, mixed 1:1 in DNA/RNA shield (Zymo Research), and stored at -80°C until quantitative PCR (qPCR) and quantitative reverse transcriptase PCR (qRT-PCR) assay. Additionally, 200 μ L of medium from the exterior of the dialysis tube was collected and stored at -80°C for NH₃ assay (Abcam, modified Berthelot) and urea assay. Sampling volumes at each time point (which were the same across all experimental groups) were not replaced as each draw represented 0.13% of the total volume per time point (which would be expected to have an insignificant impact on NH₃ and urea quantification).

TABLE 1 *Ureaplasma* isolates studied

Species	Isolate no.	Source
<i>U. parvum</i>	IDRL-10774	Bronchoalveolar lavage fluid
<i>U. parvum</i>	IDRL-11887	Bronchoalveolar lavage fluid
<i>U. parvum</i>	IDRL-11264	Sputum
<i>U. parvum</i>	ATCC-27815	Urethritis, serovar 3
<i>U. urealyticum</i>	IDRL-10763	Bronchial washings
<i>U. urealyticum</i>	IDRL-10612	Bronchoalveolar lavage fluid
<i>U. urealyticum</i>	IDRL-10611	Bronchoalveolar lavage fluid
<i>U. urealyticum</i>	IDRL-11235	Tracheal secretions
<i>U. urealyticum</i>	IDRL-12698	Bronchoalveolar lavage fluid
<i>U. urealyticum</i>	ATCC-27816	Urethritis, serovar 4

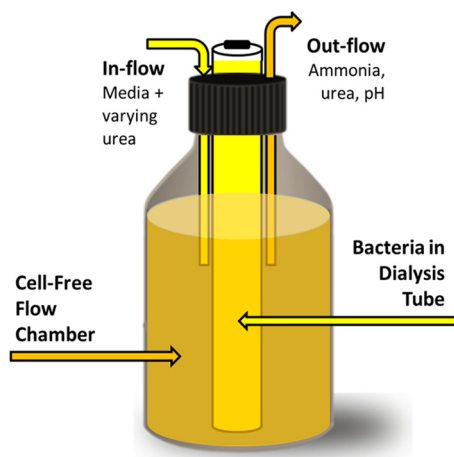


FIG 5 Schematic of the dialyzed flow system.

Experimental mouse model. C3H male and female mice (18 to 22 g; Charles River Laboratories, Wilmington, MA) were pharmacologically immunosuppressed for 7 days with methylprednisone, tacrolimus, and mycophenolate mofetil; half of the animals were administered 40 g/L urea *ad libitum* in their drinking water beginning 10 days prior to infection, resulting in an average blood urea nitrogen level (BUN) of 38 mg/dL (SD = 14) for urea-fed mice and 28 mg/dL (SD = 6.97) for control mice (unpaired *t* test: $P = 0.0156$). For IT challenge, the mice were anesthetized with ketamine/xylazine (90/10 mg/kg); 50 μ L of bacterial suspension (selected to ensure ureaplasma entry into the airways as used in our prior work [11, 12]) was placed into their trachea using a 22-gauge curved gavage needle, after which the animals were placed in a vertical position for 10 min using a murine vertical stabilization apparatus (Fig. S1). For IP challenge, 100 μ L of 10^7 to 10^8 CFU/mL bacterial suspension was injected into the peritoneum. After 24 h of infection, the animals were sacrificed, their blood was collected for NH_3 measurement with a point-of-care meter (Woodley Equipment Company Ltd., WD5502 PocketChem BA analyzer), blood CFU measurement (dilution and plating), and *U. urealyticum ureC* qPCR, and their lungs were harvested for total lung CFU measurement and *U. urealyticum ureC* qPCR. A total of 12 mice were assigned to each experimental group. A sample size of 12 mice per group yields 80% power to detect a difference of 1.75 standard deviations or larger for levels of NH_3 between two groups using two sample *t* tests.

Ethics statement. This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health and was approved by Mayo Clinic Institutional Animal Care and Use Committee (protocol number A5004-20). The Mayo Clinic is Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC) accredited (000717), registered with the U.S. Department of Agriculture (41-R-0006), and has an Assurance with the Office of Laboratory Animal Welfare (A3291-01). The mice were housed in a biosafety level 2, specific-pathogen-free, AAALAC-accredited facility. Sentinel mice were tested quarterly for murine pathogens; all were negative throughout the course of this study. The mice had *ad libitum* access to irradiated rodent food (LabDiet formula 5053) and water. The facility was environmentally controlled (temperature, 68 to 74°F; relative humidity, 30 to 70%; 12:12-h light:dark cycle). All efforts were made to minimize suffering. The animals were monitored twice daily, and anesthetized mice were monitored until awake. Mice were monitored for decreased activity, decreased body temperature, hunched stature, distress, and inability to eat and drink; if these occurred and were severe, the animals were humanely euthanized.

qPCR and qRT-PCR assays. Nucleic acids were purified from tissue and culture material using a Maxwell RSC (Promega, Madison WI). DNA isolation was carried out with a Maxwell RSC tissue DNA kit and RNA isolated with a Maxwell RSC simplyRNA tissue kit as per the manufacturer's instructions. Sample input for both kits was 100 μ L, and elution output was 100 μ L for DNA and 50 μ L for RNA.

The quantitative PCR assay for *U. parvum* and *U. urealyticum* has been previously described (39) and was performed with slight modifications to adapt the assay for use on the LightCycler 2.0 instrument. Briefly, 1 \times LightCycler DNA Master HybProbe was combined with 1 \times primer/probe set 1408 (TIB Molbiol, Howell Township, NJ) and an additional 3 mM MgCl_2 . A total of 15 μ L of the complete master mix was combined with 5 μ L of DNA extract in a LightCycler sample capillary. Thermocycling conditions were as follows: Denaturation at 95°C for 10 min; amplification for 45 cycles of 10 s at 95°C, 15 s at 60°C (single acquisition), and 15 s at 72°C; melting curve analysis/amplicon detection for 0 s at 95°C, 20 s at 59°C, 20 s at 40°C (ramp rate of 0.2°C/s), 0 s at 80°C (ramp rate of 0.2°C/s and continuous acquisition); and cooling for 30 s at 40°C.

The qRT-PCR assay was adapted from the above qPCR assay to include a RT-specific reverse primer (5'-TTGNTCAAANATTGGATCTTCC-3'). The complete master mix included 1 \times primer/probe set 1408, 0.25 mM RT-specific reverse primer, 1 \times SuperScript III Platinum One-Set qRT-PCR system buffer, 0.05 \times SuperScript enzyme, and 1 mM MgCl_2 (Invitrogen, Waltham, MA). 15 μ L of the complete RT master mix was combined with 5 μ L of RNA extract in a LightCycler sample capillary. The thermocycling conditions were as follows: reverse transcription/denaturation at 55°C for 15 min followed by 95°C for 2 min; amplification for 45 cycles of 10 s at 95°C, 20 s at 60°C (single acquisition), and 20 s at 72°C; and cooling for

30 s at 40°C. Plasmid control material (30-8706-01 *U. urealyticum* and 30-8706-03 *U. parvum*; TIB Molbiol) were serially diluted and used to generate quantification lines for each species. A single, 1×10^3 midpoint dilution of was included as a calibration control. Prequantified culture material preserved in DNA/RNA shield was included as a process control and carried through extraction and assayed with each experiment.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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REFERENCES

- Lichtenstein GR, Yang YX, Nunes FA, Lewis JD, Tuchman M, Tino G, Kaiser LR, Palevsky HI, Kotloff RM, Furth EE, Bavaria JE, Stecker MM, Kaplan P, Berry GT. 2000. Fatal hyperammonemia after orthotopic lung transplantation. *Ann Intern Med* 132:283–287. <https://doi.org/10.7326/0003-4819-132-4-200002150-00006>.
- Krutsinger D, Pezzulo A, Blevins AE, Reed RM, Voigt MD, Eberlein M. 2017. Idiopathic hyperammonemia after solid organ transplantation: primarily a lung problem? A single-center experience and systematic review. *Clin Transplant* 31:e12957. <https://doi.org/10.1111/ctr.12957>.
- Braissant O, McLin VA, Cudalbu C. 2013. Ammonia toxicity to the brain. *J Inher Metab Dis* 36:595–612. <https://doi.org/10.1007/s10545-012-9546-2>.
- Eid T, Lee T-SW. 2013. Reassessing the role of astrocytes in ammonia neurotoxicity. *Nat Med* 19:1572–1574. <https://doi.org/10.1038/nm.3420>.
- Lichtenstein GR, Kaiser LR, Tuchman M, Palevsky HI, Kotloff RM, O'Brien CB, Furth EE, Raps EC, Berry GT. 1997. Fatal hyperammonemia following orthotopic lung transplantation. *Gastroenterology* 112:236–240. [https://doi.org/10.1016/S0016-5085\(97\)70240-3](https://doi.org/10.1016/S0016-5085(97)70240-3).
- Yoshida EM, Ostrow DN, Erb SR, Fradet G. 1997. Hyperammonemia after heart-lung transplantation. *Gastroenterology* 112:2162. <https://doi.org/10.1053/gast.1997.v112.agast972162>.
- Rueda JF, Caldwell C, Brennan DC. 1998. Successful treatment of hyperammonemia after lung transplantation. *Ann Intern Med* 128:956–957. <https://doi.org/10.7326/0003-4819-128-11-199806010-00022>.
- Moffatt-Bruce SD, Pesavento T, Von Viger J, Nunley D, Pope-Harman A, Martin S, Ross P. 2008. Successful management of immunosuppression in a patient with severe hyperammonemia after lung transplantation. *J Heart Lung Transplant* 27:801–803. <https://doi.org/10.1016/j.healun.2008.03.019>.
- Chen C, Bain KB, Iuppa JA, Yusen RD, Byers DE, Patterson GA, Trulock EP, Hachem RR, Witt CA. 2016. Hyperammonemia syndrome after lung transplantation: a single center experience. *Transplantation* 100:678–684. <https://doi.org/10.1097/TP.0000000000000868>.
- Bharat A, Cunningham SA, Scott Budinger GR, Kreisel D, DeWet CJ, Gelman AE, Waites K, Crabb D, Xiao L, Bhorade S, Ambalavanan N, Dilling DF, Lowery EM, Astor T, Hachem R, Krupnick AS, DeCamp MM, Ison MG, Patel R. 2015. Disseminated *Ureaplasma* infection as a cause of fatal hyperammonemia in humans. *Sci Transl Med* 7:284re3. <https://doi.org/10.1126/scitranslmed.aaa8419>.
- Wang X, Greenwood-Quaintance KE, Karau MJ, Block DR, Mandrekar JN, Cunningham SA, Mallea JM, Patel R. 2017. *Ureaplasma parvum* causes hyperammonemia in a pharmacologically immunocompromised murine model. *Eur J Clin Microbiol Infect Dis* 36:517–522. <https://doi.org/10.1007/s10096-016-2827-1>.
- Wang X, Karau MJ, Greenwood-Quaintance KE, Block DR, Mandrekar JN, Cunningham SA, Patel R. 2016. *Ureaplasma urealyticum* causes hyperammonemia in an experimental immunocompromised murine model. *PLoS One* 11:e0161214. <https://doi.org/10.1371/journal.pone.0161214>.
- Romano N, La Licata R, Russo Alesi D. 1986. Energy production in *Ureaplasma urealyticum*. *Pediatr Infect Dis* 5:5308-12. <https://doi.org/10.1097/00006454-198611010-00024>.
- Smith DG, Russell WC, Ingledew WJ, Thirkell D. 1993. Hydrolysis of urea by *Ureaplasma urealyticum* generates a transmembrane potential with resultant ATP synthesis. *J Bacteriol* 175:3253–3258. <https://doi.org/10.1128/jb.175.11.3253-3258.1993>.
- Dando SJ, Sweeney EL, Knox CL. 2019. *Ureaplasma*, p 1–28, *Bergey's manual of systematics of archaea and bacteria*. John Wiley & Sons, Hoboken, NJ.
- Kiberenge RK, Lam H. 2015. Fatal hyperammonemia after repeat renal transplantation. *J Clin Anesth* 27:164–167. <https://doi.org/10.1016/j.jclinane.2014.09.008>.
- Beeton ML. 2016. Possible missed diagnosis of *Ureaplasma* spp infection in a case of fatal hyperammonemia after repeat renal transplantation. *J Clin Anesth* 33:504–505. <https://doi.org/10.1016/j.jclinane.2015.08.019>.

18. Navaneethan U, Venkatesh PG. 2010. Idiopathic hyperammonemia in a patient with total pancreatectomy and islet cell transplantation. *JOP* 11: 620–624.
19. Espinos J, Rifon J, Perez-Calvo J, Nieto Y. 2006. Idiopathic hyperammonemia following high-dose chemotherapy. *Bone Marrow Transplant* 37:899. <https://doi.org/10.1038/sj.bmt.1705346>.
20. Mitchell RB, Wagner JE, Karp JE, Watson AJ, Brusilow SW, Przepiorka D, Storb R, Santos GW, Burke PJ, Saral R. 1988. Syndrome of idiopathic hyperammonemia after high-dose chemotherapy: review of nine cases. *Am J Med* 85:662–667. [https://doi.org/10.1016/s0002-9343\(88\)80239-0](https://doi.org/10.1016/s0002-9343(88)80239-0).
21. Davies SM, Szabo E, Wagner JE, Ramsay NK, Weisdorf DJ. 1996. Idiopathic hyperammonemia: a frequently lethal complication of bone marrow transplantation. *Bone Marrow Transplant* 17:1119–1125.
22. Sharp RA, Lang CC. 1987. Hyperammonaemic encephalopathy in chronic myelomonocytic leukaemia. *Lancet* 1:805. [https://doi.org/10.1016/S0140-6736\(87\)92827-3](https://doi.org/10.1016/S0140-6736(87)92827-3).
23. Watson AJ, Chambers T, Karp JE, Risch VR, Walker WG, Brusilow SW. 1985. Transient idiopathic hyperammonemia in adults. *Lancet* 2:1271–1274. [https://doi.org/10.1016/s0140-6736\(85\)91554-5](https://doi.org/10.1016/s0140-6736(85)91554-5).
24. Ho AY, Mijovic A, Pagliuca A, Mufti GJ. 1997. Idiopathic hyperammonemia syndrome following allogeneic peripheral blood progenitor cell transplantation (allo-PBPCT). *Bone Marrow Transplant* 20:1007–1008. <https://doi.org/10.1038/sj.bmt.1701003>.
25. Frere P, Canivet JL, Gennigens C, Rebeix JP, Fillet G, Beguin Y. 2000. Hyperammonemia after high-dose chemotherapy and stem cell transplantation. *Bone Marrow Transplant* 26:343–345. <https://doi.org/10.1038/sj.bmt.1702485>.
26. Tse N, Cederbaum S, Glaspy JA. 1991. Hyperammonemia following allogeneic bone marrow transplantation. *Am J Hematol* 38:140–141. <https://doi.org/10.1002/ajh.2830380213>.
27. Uygun V, Karasu G, Daloğlu H, Hazar V, Yeşilipek A. 2015. Idiopathic hyperammonemia after hematopoietic stem cell transplantation: a case report. *Pediatr Transplant* 19:E104–E105. <https://doi.org/10.1111/ptr.12467>.
28. Snyder MJ, Bradford WD, Kishnani PS, Hale LP. 2003. Idiopathic hyperammonemia following an unrelated cord blood transplant for mucopolysaccharidosis I. *Pediatr Dev Pathol* 6:78–83. <https://doi.org/10.1007/s10024-001-0271-3>.
29. Watanabe R, Okamoto S, Asahi A, Mori T, Takayama N, Ikeda Y. 2000. Idiopathic hyperammonemia following allogeneic bone marrow transplantation for refractory lymphoma. *Jap J Clin Hematol* 41:1285–1288.
30. Placone N, Kao RL, Kempert P, Ruiz ME, Casillas JN, Okada M, Gibson JB, Maggi C, O'Brien K, Nattiv R, Gallant NM, Abrams SH. 2020. Hyperammonemia from *Ureaplasma* infection in an immunocompromised child. *J Pediatr Hematol Oncol* 42:e114–e116. <https://doi.org/10.1097/MPH.0000000000001414>.
31. Jacques F, El-Hamamsy I, Fortier A, Maltais S, Perrault LP, Liberman M, Noisieux N, Ferraro P. 2012. Acute renal failure following lung transplantation: risk factors, mortality, and long-term consequences. *Eur J Cardiothorac Surg* 41:193–199. <https://doi.org/10.1016/j.ejcts.2011.04.034>.
32. Lertjitbanjong P, Thongprayoon C, Cheungpasitporn W, O'Corragain OA, Srivali N, Bathini T, Watthanasuntorn K, Aeddula NR, Salim SA, Ungprasert P, Gillaspie EA, Wijarnpreecha K, Mao MA, Kaewput W. 2019. Acute kidney injury after lung transplantation: a systematic review and meta-analysis. *J Clin Med* 8:1713. <https://doi.org/10.3390/jcm8101713>.
33. Neyrolles O, Ferris S, Behbahani N, Montagnier L, Blanchard A. 1996. Organization of *Ureaplasma urealyticum* urease gene cluster and expression in a suppressor strain of *Escherichia coli*. *J Bacteriol* 178:647–655. <https://doi.org/10.1128/jb.178.3.647-655.1996>.
34. George TJ, Arnaoutakis GJ, Beaty CA, Pipeling MR, Merlo CA, Conte JV, Shah AS. 2012. Acute kidney injury increases mortality after lung transplantation. *Ann Thorac Surg* 94:185–192. <https://doi.org/10.1016/j.athoracsur.2011.11.032>.
35. Monnier A, Krummel T, Collange O, Haffner G, Hirschi S, Dégot T, Hannedouche T, Kessler R. 2015. Prevalence of acute and chronic renal failure after lung transplantation. *J Heart Lung Transplant* 34: S261. <https://doi.org/10.1016/j.healun.2015.01.726>.
36. Hanke N, Clajus C, Fegbeutel C, Walsemann D, Simon A, Haller H, Haverich A, Gueler F. 2012. Acute kidney injury after heart- and/or lung transplantation: retrospective analysis of incidence, risk factors and outcome of 1200 patients. *Transplantation* 94:2221.
37. Kenny GE, Cartwright FD. 1977. Effect of urea concentration on growth of *Ureaplasma urealyticum* (T-strain mycoplasma). *J Bacteriol* 132:144–150. <https://doi.org/10.1128/jb.132.1.144-150.1977>.
38. Fleming D, Karau M, Patel R. 2021. A novel bioreactor for the stable growth of *Ureaplasma parvum* and *Ureaplasma urealyticum*. *J Microbiol Methods* 181:106131. <https://doi.org/10.1016/j.mimet.2020.106131>.
39. Cunningham SA, Mandrekar JN, Rosenblatt JE, Patel R. 2013. Rapid PCR detection of *Mycoplasma hominis*, *Ureaplasma urealyticum*, and *Ureaplasma parvum*. *Int J Bacteriol* 2013:168742. <https://doi.org/10.1155/2013/168742>.