

IPSE, a Urogenital Parasite-Derived Immunomodulatory Molecule, Suppresses Bladder Pathogenesis and Anti-Microbial Peptide Gene Expression in Bacterial Urinary Tract Co-Infection

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Abstract

Background: Parasitic infections can increase susceptibility to bacterial co-infections. This may be true for urogenital schistosomiasis and bacterial urinary tract co-infections (UTI). We previously reported that this co-infection is facilitated by *S. haematobium* eggs triggering interleukin-4 (IL-4) production and sought to dissect the underlying mechanisms.

The interleukin-4 inducing principle from *Schistosoma mansoni* eggs (IPSE) is one of the most abundant schistosome egg-secreted proteins and binds to IgE on the surface of basophils and mast cells to trigger IL-4 release. IPSE can also translocate into host nuclei using a nuclear localization sequence (NLS) to modulate host transcription. We hypothesized that IPSE is the factor responsible for the ability of *S. haematobium* eggs to worsen UTI pathogenesis.

Methods: Mice were intravenously administered a single 25 µg dose of recombinant *S. haematobium*-derived IPSE, an NLS mutant of IPSE or PBS. Following IPSE exposure, mice were serially weighed, and organs analyzed by histology to assess for toxicity. Twenty-four hours after IPSE administration, mice were challenged with the uropathogenic *E. coli* strain UTI89 by urethral catheterization. Bacterial cfu were measured using urine. Bladders were examined histologically for UTI-triggered pathogenesis and by PCR for antimicrobial peptide expression.

Results: Unexpectedly, IPSE administration did not result in significant differences in urine bacterial CFU. However, IPSE administration did lead to a significant reduction in UTI-induced bladder pathogenesis and the expression of anti-microbial peptides in the bladder. Despite the profound effect of IPSE on UTI-triggered bladder pathogenesis and anti-microbial peptide production, mice did not demonstrate systemic ill effects from IPSE exposure.

Conclusions: Our data show that IPSE may play a major role in *S. haematobium*-associated urinary tract co-infection, albeit in an unexpected fashion. These findings also indicate that IPSE either works in concert with other IL-4-inducing factors to increase susceptibility of *S. haematobium*-infected hosts to bacterial co-infection, or does not contribute to enhancing vulnerability to this co-infection.

Background

Urogenital schistosomiasis, infection of the urogenital tract by *Schistosoma* helminths, is primarily caused by *Schistosoma haematobium*. This infection afflicts approximately 112 million people, in other words the majority of the approximately 200 million people worldwide with schistosomiasis (1). Bacterial urinary tract infections (UTI) are even more prevalent, affecting half of all girls and women at least once in their lifetimes (2). Several reports have observed that patients with urogenital schistosomiasis are more prone to bacterial UTI (3–5), although two papers have disputed this association (6, 7). The potential causal link between urogenital schistosomiasis and UTI has been debated. One hypothesis is that *S. haematobium* infection leads to immune polarization that is detrimental to clearance of bacteriuria. Indeed, we have demonstrated in a mouse model that *S. haematobium* eggs injected into the

bladder wall (a primary tissue site containing eggs in patients with urogenital schistosomiasis) increase susceptibility to subsequent UTI (8). This increased susceptibility is associated with egg-induced, IL-4-driven suppression of natural killer T (NKT) cell activity. The role of NKT cells was confirmed by restoring the activity of this cellular subset using α -galactosylceramide, an NKT cell agonist. Thus, the heightened vulnerability of patients with urogenital schistosomiasis to UTI may be immunologically mediated.

The egg-associated factors which orchestrate this IL-4-dependent phenomenon may include the interleukin-4-inducing principle of *Schistosoma mansoni* eggs (IPSE)(9), also known as α -1(10). IPSE is one of the most abundant schistosome egg-secreted proteins (11), and features multiple immunomodulatory properties. First, IPSE binds to Fc ϵ receptor-bound IgE on the surface of basophils and mast cells to trigger IL-4 secretion (12–14). It is also able to ligate immunoglobulins on the surface of B regulatory cells (Bregs) and subsequently activate these cells (15). The *S. mansoni* ortholog of IPSE named *S. mansoni* chemokine-binding protein (smCKBP) can neutralize chemokines (16). Lastly, IPSE features a nuclear localization sequence which guides the protein to host cell nuclei (17, 18), where it modulates transcription (19, 20).

We hypothesized that IPSE may be the key IL-4-inducing principle of *S. haematobium* eggs responsible for increased UTI susceptibility in affected hosts. Our interest in testing this hypothesis was based both on a wish to better understand the pathogenesis of urogenital schistosomiasis as well as a desire to learn the safety profile of IPSE as a potential therapeutic. In past work we have demonstrated that IPSE is a potent anti-inflammatory agent for ifosfamide-induced hemorrhagic cystitis (12, 20, 21). We are currently investigating the use of IPSE for other types of bladder inflammation.

Herein we report that IPSE neither affected levels of bacteriuria nor infection-associated pain in UTI-challenged mice. However, IPSE exposure did lead to markedly reduced UTI-triggered bladder pathogenesis and a decrease in expression of anti-microbial peptides. Finally, IPSE was not associated with any discernable adverse systemic effects.

Methods

Study approval

All animal work was conducted according to relevant U.S. and international guidelines. Specifically, animal experimental work was reviewed and approved as protocol 14 – 03 by the Institutional Animal Care and Use Committee of the Biomedical Research Institute (Rockville, Maryland, USA). Our Institutional Animal Care and Use Committee guidelines comply with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Mice

Female 6- to 7-wk-old BALB/c mice (Charles River Laboratories, Wilmington, MA, USA) were housed under 12 h light- dark cycles in temperature-controlled holding rooms with unlimited access to dry mouse chow

and water. Newly received mice were acclimated to the animal facility for at least one week prior to experimental use.

IPSE protein production and labeling

Recombinant H06 H-IPSE (one of the major *Schistosoma haematobium* IPSE orthologs) and an NLS mutant of H06 H-IPSE (the wild type NLS sequence SKRGRKY changed to SAAGAAY) were produced in HEK293-6E cells as previously described (18). H06 IPSE was conjugated to Alexa Fluor 488 using a Alexa Fluor 488 antibody labeling kit (ThermoFisher Scientific, Waltham, MA) according to manufacturer instructions; however, the pH was kept at 7.4 throughout the reaction to enrich for labeling of the terminal amine (pKA of 7.4). The efficiency of conjugation was confirmed by Nanodrop. The typical degree of labeling was one mole of dye per mole of IPSE, which suggested IPSE was only labeled on the terminal amine. Avoiding labelling of lysine or arginine residues in the protein backbone is critical, as each of the positively charged amino acids in the NLS is essential for nuclear translocation(17). Low labeling efficiently thus minimized the potential interference of the dye with IPSE's functional domains.

IPSE administration

One day prior to UTI induction, mice underwent tail vein injection with phosphate-buffered saline or 25 µg of H06 H-IPSE (or its NLS mutant) in phosphate-buffered saline.

S. haematobium egg injection

Bladder wall injections were performed as described previously (22). Female BALB/c mice were anesthetized with isoflurane, a midline lower abdominal incision was made, and the bladder was exteriorized. Freshly prepared *S. haematobium* eggs (3,000 eggs in 50 µl of phosphate-buffered saline) or control vehicle (un-infected hamster liver and intestinal extract in 50 µl of phosphate-buffered saline) was injected submucosally into the anterior aspect of the bladder. Abdominal incisions were then closed with 4 - 0 Vicryl sutures, and the surgical site was treated once with topical antibiotic ointment.

UTI89 infection of mice

The uropathogenic *Escherichia coli* (UPEC) strain UTI89 was derived from a patient with cystitis (23). Briefly, UTI89 was streaked on an LB agar plate to obtain a single colony which was then inoculated into 10 ml of LB broth and grown at 37 °C overnight under static conditions to promote type 1-pilus expression(24, 25). Bacteria were then subcultured 1:100 into 10 ml of fresh medium, followed by growth at 37 °C for another 18 h. These cultures were centrifuged for 10 min at 14,000 rpm, resuspended in 2 ml PBS, and then diluted to approximately 2×10^9 CFU per ml as estimated using a spectrophotometer (OD_{600 nm}). 50 µl of this suspension (10^8 CFU) was inoculated into the bladders of female mice by transurethral catheterization (26).

Bacterial titer determinations

Freshly collected urine was serially diluted 100- and 1,000-fold in sterile PBS. Twenty-five microliters of each dilution was plated onto MacConkey agar plates. CFU were counted after overnight incubation at 37 °C. The limits of detection were 4,000 CFU/ml for urine.

Histology

Bladder tissues were fixed in neutral buffered formalin, dehydrated, and embedded in paraffin. Five-micrometer sections were stained with hematoxylin and eosin. Histology was analyzed for each bladder by a board-certified pathologist (JIO) in a blinded fashion, using the following scoring system:

Urothelium

0 Normal

1 urothelial hyperplasia with reactive nuclear atypia and architectural disarray

2 segmental urothelial ulceration

Edema

0 Normal

1 perivascular edema with expansion but preservation of bladder architecture

2 severe edema with effacement of the submucosa

Inflammation

0 Normal

1 perivascular acute inflammatory infiltrates

2 frank acute inflammation of the urothelium with urothelial microabscesses

Hemorrhage

0 None

1 missing?

2 frank intra-tissue hemorrhage

Contraction

0 Normal papillae

1 blunted/wide contraction papillae

2 effaced contraction papillae

0.5 gradations for focality

RNA purification

RNA was isolated from mouse bladders using TRIzol Reagent and PureLink RNA Mini Kit (Invitrogen), according to manufacturers' instructions. Briefly, aseptically excised bladders were homogenized in 1 ml TRIzol Reagent by bead-beating using ceramic beads (Omni International) and a mini-bead beater (Biospec). Following a 5-min incubation, 0.2 ml chloroform was added and again incubated for 3 min before centrifugation at $12,000 \times g$ for 15 min to separate homogenates into aqueous and organic phases. The aqueous supernatant ($\sim 400 \mu\text{l}$) was mixed with an equal volume of 70% ethanol before binding the mixture to RNA binding columns by centrifugation. On-column DNase digestion (Invitrogen) was performed for 30 minutes, following the manufacturer's protocols. After column washes and drying, RNA was eluted in RNase-free water, quantified and its quality checked using a NanoDrop 1000 spectrophotometer (Thermo Scientific) and Bioanalyzer 2100 (Agilent).

cDNA synthesis, and real-time PCR

cDNA synthesis was performed using the RT2 First Strand cDNA kit (SABiosciences). Real-time PCR was performed for anti-microbial peptide gene expression using an Mx3005p thermocycler (Stratagene) using an RT2 custom PCR array (SABiosciences) with RT2 SYBR green quantitative PCR (qPCR) master mixes (SABiosciences). Cycle thresholds (C_T) were calculated for each reaction. Using the comparative C_T method, relative gene expression was calculated as $2^{(-\Delta\Delta C_T)}$, where $\Delta C_T = C_T$ (gene of interest) - ΔC_T (β -actin). $\Delta\Delta C_T$ was calculated as ΔC_T (coinfected) - ΔC_T (bacterially monoinfected). Data are expressed as means \pm standard deviations (SDs). P values were calculated using Mann-Whitney U tests comparing ΔC_T of coexposed and bacterially monoinfected mice (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$). Melt curves confirmed the specificity of PCR reactions. PCR primer sequences are shown in Supplemental Table 1.

Toxicity assays

Toxicity assays were performed using four groups including one vehicle control (phosphate-buffered saline) and three IPSE treated groups (0.5, 1, and 2 mg/kg/day via tail vein injection daily for 7 days), using BALB/c mice. The dosing volume was kept constant at 6.35 mL/kg/day for each mouse. Parameters evaluated included clinical signs of illness, body weights, percent body weight gain, feed consumption, hematology, clinical chemistry, and after 7 days, organ weights, gross pathology, and histopathology.

Histamine measurements

Two groups of mice (BALB/c 6-week old, $n = 4$ in each group) were injected with either PBS or 25 μg IPSE intravenously. Mice were bled 5 minutes after injection with PBS or IPSE *via* cheek bleed and 30 minutes post injection *via* terminal heart bleed. Sera was collected and immediately used for histamine analysis

using a mouse histamine ELISA kit from MyBioSource using manufacturer's instructions (Catalog # MBS725193).

Statistical analysis

Statistical analyses were performed using GraphPad software. Except where noted otherwise, Mann-Whitney U tests and Student t tests were used to evaluate statistical significance for nonparametrically and parametrically distributed data, respectively. P values of < 0.05 were defined as significant.

Results

IPSE Does Not Alter Susceptibility to UTI

In prior work we have demonstrated that a single injection of *S. haematobium* eggs into the bladder walls of otherwise UTI-resistant BALB/c mice renders them much more sensitive to UTI (8). Thus, herein we sought to determine whether IPSE is sufficient to confer increased susceptibility to UTI. Surprisingly, a high intravenous dose of IPSE (25 μ g, Fig. 1) did not enhance vulnerability of mice to transurethral infection with the uropathogenic UTI89 *E. coli* strain. This was true over an entire week of daily urine sampling, and a nuclear localization sequence (NLS)-deficient mutant of IPSE also did not alter UTI susceptibility in mice.

IPSE Reduces UTI-Associated Bladder Pathology

In past work we have reported that IPSE dampens ifosfamide-triggered bladder pathogenesis. Hence, we tested whether IPSE alleviates UTI-induced bladder pathology. A single intravenous dose of IPSE indeed reduced bladder edema and inflammation, but not urothelial denudation or contraction (Fig. 2).

IPSE Reduces Expression of Anti-Microbial Peptides in the Bladder

Considering that IPSE ameliorated UTI-triggered bladder inflammation, we postulated that this molecule may also modulate bladder production of anti-microbial peptides, which are part of the inflammatory response to infections. Amongst 8 anti-microbial peptides assayed, three of them featured reduced gene expression following IPSE exposure (lipocalin, beta-defensin, and alpha-defensin-4, Fig. 3).

IPSE Does Not Increase Circulating Histamine

IPSE is known to ligate Fc ϵ receptor-bound IgE on the surface of basophils and mast cells (12–14), which could conceivably lead to histamine release and anaphylaxis. We therefore tested the influence of intravenous IPSE on circulating histamine in mice. These assays revealed that systemic histamine levels are not increased in IPSE-exposed mice at 5 or 30 minutes post-IPSE injection (Fig. 4).

IPSE Does Not Cause Systemic Pathology

Taking into account IPSE's multi-faceted immunomodulatory effects on the host, our next objective was to determine if IPSE exposure leads to systemic pathology. Intravenous administration of IPSE at doses of 0.5, 1 and 2 mg/kg/day to mice did not lead to any abnormal clinical signs in mice. All animals survived to study termination. Treatment with IPSE at doses up to 2 mg/kg/day had no effect on body weight (Tables 1 and 2), body weight gain (Tables 3 and 4), feed consumption, hematology, clinical chemistry, or gross pathology (see supplemental data). Clinical chemistry analytes of all IPSE-treated animals were comparable to control animals, except increased globulin (by 1.1 fold) and decreased albumin globulin (A:G) ratio (by 1.2 fold) at 0.5 mg/kg/day in females. Organ weights of all IPSE-treated animals were comparable to control animals, except increased relative weights of liver at 1 mg/kg/day in females. Histopathological evaluation of mice treated with IPSE did not show any lesions of pathological significance except for minimal focal basophilic tubules in kidneys in one male and one female mouse receiving either control vehicle or 2 mg/kg/day of IPSE (i.e., four mice total), minimal focal neutrophil and lymphocytes infiltration at the site of injection (one male mouse receiving vehicle and one female mouse receiving 2 mg/kg/day of IPSE), minimal focal extramedullary hematopoiesis in the liver of one vehicle-treated female mouse, minimal focal cystic spaces in the liver of one female mouse receiving 2 mg/kg/day of IPSE, and minimal unilateral atrophy of the uterus in two female mice receiving 2 mg/kg/day of IPSE.

Table 1
Summary - Body Weight (g) - Male

Mean/SD/N	Treatment Day		
	1	4	7
Group: G1Dose: 0 mg/kg/day			
Mean	22.00	22.03	21.70
SD	0.82	0.67	0.97
N	6	6	6
Group: G2Dose: 0.5 mg/kg/day			
Mean	22.13	21.85	22.20
SD	0.85	1.31	1.62
N	6	6	6
Group: G3Dose: 1 mg/kg/day			
Mean	22.33	21.92	22.28
SD	0.95	1.05	0.82
N	6	6	6
Group: G4Dose: 2 mg/kg/day			
Mean	22.48	22.12	21.72
SD	1.09	1.37	1.15
N	6	6	6
Key: N = Number of animals.			

Table 2
Summary - Body Weight (g) - Female

Mean/SD/N	Treatment Day		
	1	4	7
Group: G1Dose: 0 mg/kg/day			
Mean	18.08	17.95	17.93
SD	0.80	0.40	0.25
N	6	6	6
Group: G2Dose: 0.5 mg/kg/day			
Mean	18.25	17.93	18.32
SD	0.78	0.91	0.60
N	6	6	6
Group: G3Dose: 1 mg/kg/day			
Mean	18.37	18.07	18.28
SD	0.93	1.25	1.35
N	6	6	6
Group: G4Dose: 2 mg/kg/day			
Mean	18.55	18.02	18.17
SD	0.89	0.63	0.86
N	6	6	6
Key: N = Number of animals.			

Table 3
 Summary - Body Weight Gain (%) -
 Male

Mean/SD/N	Treatment Day	
	1 to 4	1 to 7
Group: G1Dose: 0 mg/kg/day		
Mean	0.18	-1.38
SD	1.29	1.15
N	6	6
Group: G2Dose: 0.5 mg/kg/day		
Mean	-1.30	0.21
SD	4.18	4.40
N	6	6
Group: G3Dose: 1 mg/kg/day		
Mean	-1.87	-0.17
SD	1.71	2.75
N	6	6
Group: G4Dose: 2 mg/kg/day		
Mean	-1.65	-3.40
SD	3.18	2.45
N	6	6
Key: N = Number of Animals.		

Table 4
Summary - Body Weight Gain (%) -
Female

Mean/SD/N	Treatment Day	
	1 to 4	1 to 7
Group: G1Dose: 0 mg/kg/day		
Mean	-0.63	-0.71
SD	3.30	3.22
N	6	6
Group: G2Dose: 0.5 mg/kg/day		
Mean	-1.74	0.42
SD	2.34	2.52
N	6	6
Group: G3Dose: 1 mg/kg/day		
Mean	-1.68	-0.53
SD	3.21	3.12
N	6	6
Group: G4Dose: 2 mg/kg/day		
Mean	-2.75	-1.94
SD	4.32	5.35
N	6	6
Key: N = Number of Animals.		

Discussion

Helminths have co-evolved with humans to immunomodulate their hosts. This includes schistosomes, which affect at least 200 million people in Brazil, sub-Saharan Africa, the Middle East, and Asia (1). The main schistosomes affecting humans include *Schistosoma mansoni*, *Schistosoma haematobium*, and *Schistosoma japonicum*, with *S. haematobium* comprising the majority of infections. All three of these species secrete immunomodulatory proteins, such as omega-1 (27), kappa-5 (28), SJHME-1 (29), Sj16 (30), and the interleukin-4-inducing principle of *Schistosoma mansoni* eggs (IPSE) (9), also called α -1(10). These immunomodulatory products of schistosomes have been postulated to exert far-reaching regulatory effects on host responses to not only schistosomiasis, but also co-infections.

Schistosomiasis is co-endemic with many other pathogens, including *Plasmodium* (31), HIV (31), mycobacteria (32), other helminths and parasites (33), and also *Enterobacteriaceae* such as *Salmonella* (34). Another member of *Enterobacteriaceae* that may co-infect large numbers of patients with schistosomiasis is *E. coli*.

Of the various types of disease-causing *E. coli*, uropathogenic strains are a common cause of infection in male and females, ranging from neonates to the elderly. One-quarter of all girls and women will experience multiple bacterial urinary tract infections (UTI) (35). Three different publications have linked urogenital schistosomiasis with bacterial UTI (3–5). However, two other papers have debated whether there is a causal connection between these infections (6, 7). There are multiple hypotheses that could explain a potential cause-and-effect relationship between *S. haematobium* infection, the primary cause of urogenital schistosomiasis, and UTI. It is possible, for instance, that detection bias contributes to increased reporting of UTI among patients with urogenital schistosomiasis. In this scenario, increased urine testing and urinary symptom tracking of individuals living in endemic areas leads to incidental discovery of UTI. Another potential hypothesis explaining a causal link between urogenital schistosomiasis and UTI is related to urinary tract obstruction. Schistosome egg deposition in the bladder initiates granulomatous fibrosis. In severe cases, this fibrosis can obstruct the urinary tract, contributing to urine stasis and bacterial overgrowth. Finally, it is possible that *S. haematobium* infection leads to immune skewing that hinders host clearance of bacteriuria. In support of this theory, we showed that *S. haematobium* eggs injected into the mouse bladder wall renders BALB/c mice, which are typically UTI-resistant, highly sensitive to subsequent UTI (8). The ability of *S. haematobium* eggs to enhance susceptibility to urinary tract co-infection relies upon IL-4, and also depended upon this cytokine dampening NK T cell activity. These findings lend credence to the possibility that the increased sensitivity of patients with urogenital schistosomiasis to UTI is orchestrated by schistosome-based immune modulation.

The egg-associated mediators which coordinate this IL-4-dependent phenomenon may include IPSE. This molecule is secreted by schistosome eggs in large amounts (11), and exhibits numerous immunomodulatory features. IPSE received its moniker because it ligates IgE- Fc ϵ receptor complexes on the surface of basophils and mast cells and thereby induces IL-4 secretion (12–14). In a presumably similar manner, it activates B regulatory cells (Bregs) (15). The *S. mansoni* ortholog of IPSE called *S. mansoni* chemokine-binding protein (smCKBP) can sequester a number of chemokines (16). In addition, IPSE contains a nuclear localization sequence which traffics the protein to host cell nuclei (17, 18), where it alters transcription (19, 20).

Based on IPSE's capacity to trigger IL-4 secretion and modulate host immunity more broadly, we posited that mouse exposure to *S. haematobium*-derived IPSE would be sufficient to augment UTI sensitivity. We sought to examine this postulate for two reasons. Firstly, we wished to delineate IPSE's immune mechanisms given its association with urogenital schistosomiasis. We also sought to ascertain whether and how IPSE would be efficacious against detrimental, UTI-associated bladder inflammation. This goal

was based on our past observations that a single injection of IPSE prevents severe bladder inflammation triggered by ifosfamide exposure (12, 20, 21).

Taking into account IPSE's profound effects on other types of bladder inflammation, we were surprised by our finding that IPSE did not alter clearance of subsequent UTI. Specifically, IPSE did not change mean bacterial cfu over a week of daily urine sampling. IPSE also did not modify the distribution of bacterial cfu; in the mouse UTI model, sometimes mice in the same treatment group can cluster into bimodal distributions in terms of magnitude of bacteriuria. Monitoring levels of bacteriuria serially over multiple time points is important considering that the temporal kinetics of the mouse UTI model are highly dynamic. Our findings indicate that despite IPSE's multi-faceted effects on host immunity, this molecule neither helps nor hinders clearance of bacteriuria.

Although IPSE did not affect bacteriuria, it did alter UTI-induced bladder pathology. Namely, IPSE decreased bladder inflammation and edema, but did not ameliorate bladder contraction or urothelial denudation. By comparison, we have previously shown that H06-IPSE, the same ortholog tested in this study, reduces ifosfamide-induced bladder hemorrhage and histologically evident edema, but not bladder wet weights (12). In another publication we observed that H03-IPSE, another major *S. haematobium* ortholog, dampens ifosfamide-induced bladder wet weight increases (21). We did not measure bladder hemorrhage and wet weights in this study because bacterial UTI does not significantly alter these parameters (data not shown). Certainly, IPSE's alleviation of UTI-triggered bladder inflammation is consistent with its known immunomodulatory properties.

After observing IPSE's effects on UTI-associated bladder inflammation, we measured this molecule's impact on bladder expression of anti-microbial peptides, an important set of immune responses to infection that evolved in early eukaryotes. Of the eight anti-microbial peptides examined, gene expression of lipocalin, beta-defensin, and alpha-defensin-4 were decreased by IPSE exposure. Lipocalin expression is associated with urinary tract infection (36). Transfection of the bladder urothelium with beta-defensin results in less mucosal damage, edema, and inflammation following UTI induction (37). These observations may appear puzzling when considered in the context of our findings; IPSE-mediated decreases in beta-defensin would thus be thought to enhance bladder damage. However, the functions of anti-microbial peptides are highly redundant, as a number of single anti-microbial peptide gene knockout mice, including beta-defensin-1 (38), have not demonstrated impressive UTI-associated phenotypes (39). Moreover, inhibition of certain anti-microbial peptides in the setting of UTI has been associated with less bladder damage (40). Thus, the effects of anti-microbial peptides on the bladder are complex. Finally, to our knowledge, alpha-defensin-4 does not have a critical role in UTI-associated immune responses.

Besides effects on anti-microbial peptide gene expression, IPSE has influence over basophil and mast cell immune responses. This molecule binds to Fcε receptor-associated IgE on the surface of basophils and mast cells (12–14). Ligation of these complexes could trigger histamine release and anaphylaxis, which are thought to have evolved as part of immune responses to parasites and their products (reviewed by Huang et al. (41)). Understanding IPSE's ability to trigger histamine release is important to both

comprehension of the pathobiology of schistosomiasis and defining this molecule's safety profile as a potential therapeutic. When IPSE was intravenously administered to mice, systemic histamine levels were not elevated at either 5 or 30 minutes post injection. The 5-minute time point is particularly noteworthy considering that histamine release through anaphylactic degranulation is a very rapid physiologic process.

We also sought to characterize systemic aspects of host exposure to IPSE besides histamine release. In a repeated daily dose titration of intravenous IPSE at up to 2 mg/kg/day, we did not note any obvious adverse effects in terms of body weight trends, feed consumption, hematology, clinical chemistry, or gross pathology. There were very mild alterations in globulin levels and female liver weights (1 mg/kg/day of IPSE) that were not clearly significant. We observed sporadic, minimal changes in the kidneys, injection sites, and liver in some animals, but many of these changes were also seen in control animals.

Our findings have noteworthy caveats. For instance, the dosing of IPSE may not have been physiologic. Although IPSE is one of the most abundant egg-secreted proteins, it is difficult to estimate the exact tissue concentrations of IPSE that a *S. haematobium*-infected host would feature. Histamine responses, as well as sensitivity to histamine, are different in mice versus humans. Namely, humans are much more sensitive to histamine than mice. Furthermore, murine basophils have a tenfold lower histamine content (0.1 pg vs 1 pg/cell) compared with their human counterparts (42). Although we attempted to perform a thorough assessment of any potential systemic pathology caused by IPSE exposure, it is possible that an important endpoint was not measured. In addition, differential binding affinities of IPSE for IgG and IgE could explain why there is no systemic anaphylactic degranulation. IPSE may bind to IgE with higher affinity than to IgG, but given there is several thousand fold excess of IgG in blood, IPSE may be "saturated" with IgG and cannot engage IgE on basophils.

In conclusion, IPSE is not sufficient to induce increased susceptibility to bacterial urinary tract co-infection. This suggests that IPSE is either not involved in potential enhanced vulnerability of patients with urogenital schistosomiasis to bacterial urinary tract co-infection, or that it works in concert with additional immunomodulatory factors produced by *S. haematobium*. IPSE's apparent lack of toxicity and ability to alleviate UTI-triggered bladder inflammation and edema indicates that it may be suitable for continued development as a potential therapeutic for bladder inflammatory disorders.

List Of Abbreviations

A:G, albumin globulin ratio

C_T, Cycle thresholds

IgE, immunoglobulin E

IL-4, interleukin-4

IPSE, Interleukin-4 inducing principle from *Schistosoma mansoni* eggs

LB, Luria-Bertani

NKT, natural killer T

NLS, nuclear localization sequence

PBS, phosphate-buffered saline

PCR, polymerase chain reaction

smCKBP, *S. mansoni* chemokine-binding protein

SD, standard deviation

UTI, urinary tract (co-)infections

UPEC, uropathogenic *Escherichia coli*

Declarations

Ethics approval and consent to participate

All animal work was conducted according to relevant U.S. and international guidelines. Specifically, animal experimental work was reviewed and approved as protocol 14-03 by the Institutional Animal Care and Use Committee of the Biomedical Research Institute (Rockville, Maryland, USA). Our Institutional Animal Care and Use Committee guidelines comply with the U.S. Public Health Service Policy on Humane Care and Use of Laboratory Animals.

Consent for publication

All authors grant consent for publication of this manuscript.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

ECM designed, conducted, and interpreted mouse and PCR studies. LL conducted and interpreted histamine assays. LFP assisted with IPSE protein production and purification. JIO interpreted bladder histology. KL conducted PCR studies. TSJ and FHF supervised IPSE protein production, purification and quality controls. MHH provided overall experimental design guidance, data interpretation, and wrote the manuscript. ECM, LL, FHF, and MHH participated in editing the manuscript.

References

1. Utzinger J, Raso G, Steinmann P, Zhou XN, Vounatsou P, Brooker S, et al. Schistosomiasis. In: Encyclopedia of Environmental Health. 2019.
2. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Dis Mon* [Internet]. 2003 Mar [cited 2013 May 30];49(2):53–70. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/12601337>
3. Adeyeba OAA, Ojeaga SGTGT. Urinary Schistosomiasis and Concomitant Urinary Tract Pathogens Among School Children in Metropolitan Ibadan. *Afr J Biomed Res* [Internet]. 2002;5(3):103–8. Available from: <http://www.ajol.info/index.php/ajbr/article/view/53993>
4. Laughlin LW, Farid Z, Mansour N, Edman DC, Higashi GI. Bacteriuria in urinary schistosomiasis in Egypt a prevalence survey. *Am J Trop Med Hyg* [Internet]. 1978 Sep [cited 2013 Jun 28];27(5):916–8. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/362956>
5. Uneke CJ, Ugwuoke-Adibuah S, Nwakpu KO, Ngwu BAF. An Assessment of Schistosoma haematobium infection and urinary tract bacterial infection amongst school children in rural eastern Nigeria - IS PUB. *Intern J Lab Med* [Internet]. 2009;4(1). Available from: <http://archive.ispub.com/journal/the-internet-journal-of-laboratory-medicine/volume-4-number-1/an-assessment-of-schistosoma-haematobium-infection-and-urinary-tract-bacterial-infection-amongst-school-children-in-rural-eastern-nigeria-2.html#sthash.VyTbYQ5c>
6. Eyong ME, Ikepeme EE, Ekanem EE. Relationship between Schistosoma haematobium infection and urinary tract infection among children in South Eastern, Nigeria. *Niger Postgrad Med J* [Internet]. 2008 Jun [cited 2013 Jul 8];15(2):89–93. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/18575479>
7. Pugh RN, Gilles HM. Malumfashi Endemic Diseases Research Project, X. Schistosoma haematobium and bacteriuria in the Malumfashi area. *Ann Trop Med Parasitol* [Internet]. 1979 Aug [cited 2013 Jul 8];73(4):349–54. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/496487>
8. Hsieh Y-JH, Fu C-LL, Hsieh MH. Helminth-Induced Interleukin-4 Abrogates Invariant Natural Killer T Cell Activation-Associated Clearance of Bacterial Infection. *Infect Immun* [Internet]. 2014 Mar 18 [cited 2014 Mar 20];82(5):2087–97. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/24643536>
9. Schramm G, Falcone FH, Gronow A, Haisch K, Mamat U, Doenhoff MJ, et al. Molecular characterization of an interleukin-4-inducing factor from Schistosoma mansoni eggs. *J Biol Chem*

- [Internet]. 2003/03/08. 2003 May;278(20):18384–92. Available from:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12624091
10. Dunne DW, Lucas S, Bickle Q, Pearson S, Madgwick L, Bain J, et al. Identification and partial purification of an antigen (ω 1) from schistosoma mansoni eggs which is putatively hepatotoxic in t-cell deprived mice. *Trans R Soc Trop Med Hyg.* 1981;
 11. Mathieson W, Wilson RA. A comparative proteomic study of the undeveloped and developed *Schistosoma mansoni* egg and its contents: the miracidium, hatch fluid and secretions. *Int J Parasitol* [Internet]. 2010 Apr [cited 2012 Mar 15];40(5):617–28. Available from:
<http://www.ncbi.nlm.nih.gov/pubmed/19917288>
 12. Mbanefo EC, Le L, Pennington LF, Odegaard JI, Jardetzky TS, Alouffi A, et al. Therapeutic exploitation of IPSE, a urogenital parasite-derived host modulatory protein, for chemotherapy-induced hemorrhagic cystitis. *FASEB J* [Internet]. 2018;32(8):fj.201701415R. Available from:
<http://www.fasebj.org/doi/10.1096/fj.201701415R>
 13. Knuhr K, Langhans K, Nyenhuis S, Viertmann K, Kildemoes AMO, Doenhoff MJ, et al. *Schistosoma mansoni* Egg-Released IPSE / α -1 Dampens Inflammatory Cytokine Responses via Basophil Interleukin (IL) -4 and IL-13. 2018;9(October):1–15.
 14. Meyer NH, Mayerhofer H, Tripsianes K, Blindow S, Barths D, Mewes A, et al. A crystallin fold in the interleukin-4-inducing principle of *Schistosoma mansoni* eggs (IPSE/ α -1) mediates IgE binding for antigen-independent basophil activation. *J Biol Chem* [Internet]. 2015;jbc.M115.675066. Available from: <http://www.jbc.org/lookup/doi/10.1074/jbc.M115.675066>
 15. Haeberlein S, Obieglo K, Ozir-Fazalalikhani A, Chayé MAM, Veninga H, van der Vlugt LEPM, et al. Schistosome egg antigens, including the glycoprotein IPSE/ α -1, trigger the development of regulatory B cells. *PLoS Pathog.* 2017;13(7):1–28.
 16. Smith P, Fallon RE, Mangan NE, Walsh CM, Saraiva M, Sayers JR, et al. *Schistosoma mansoni* secretes a chemokine binding protein with antiinflammatory activity. *J Exp Med* [Internet]. 2005/11/23. 2005 Nov;202(10):1319–25. Available from:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=16301741
 17. Kaur I, Schramm G, Everts B, Scholzen T, Kindle KB, Beetz C, et al. Interleukin-4-inducing principle from *Schistosoma mansoni* eggs contains a functional C-terminal nuclear localization signal necessary for nuclear translocation in mammalian cells but not for its uptake. *Infect Immun* [Internet]. 2011/01/12. 2011;79(4):1779–88. Available from:
<http://www.ncbi.nlm.nih.gov/pubmed/21220486>
 18. Pennington LF, Alouffi A, Mbanefo EC, Ray D, Heery DM, Jardetzky TS, et al. H-IPSE Is a Pathogen-Secreted Host Nucleus-Infiltrating Protein (Infiltrin) Expressed Exclusively by the *Schistosoma haematobium* Egg Stage. Appleton JA, editor. *Infect Immun* [Internet]. 2017 Dec;85(12):e00301-17. Available from: <http://iai.asm.org/lookup/doi/10.1128/IAI.00301-17>

19. Roderfeld M, Padem S, Lichtenberger J, Quack T, Weiskirchen R, Longerich T, et al. *Schistosoma mansoni* Egg-Secreted Antigens Activate Hepatocellular Carcinoma-Associated Transcription Factors c-Jun and STAT3 in Hamster and Human Hepatocytes. *Hepatology* [Internet]. 2019 Feb 12 [cited 2019 Sep 7]; Available from: <http://www.ncbi.nlm.nih.gov/pubmed/30053321>
20. Mbanefo EC, Le L, Zee R, Banskota N, Ishida K, Pennington LF, et al. IPSE, a urogenital parasite-derived immunomodulatory protein, ameliorates ifosfamide-induced hemorrhagic cystitis through downregulation of pro-inflammatory pathways. *Sci Rep* [Internet]. 2019;9(1):1–13. Available from: <http://dx.doi.org/10.1038/s41598-018-38274-z>
21. Zee RS, Mbanefo EC, Le LH, Pennington LF, Odegaard JI, Jardetzky TS, et al. IPSE, a parasite-derived host immunomodulatory protein, is a potential therapeutic for hemorrhagic cystitis. *Am J Physiol Physiol* [Internet]. 2019 Jun 1;316(6):F1133–40. Available from: <https://www.physiology.org/doi/10.1152/ajprenal.00468.2018>
22. Fu C-LL, Apelo CA, Torres B, Thai KH, Hsieh MH. Mouse bladder wall injection. *J Vis Exp* [Internet]. 2011/07/22. 2011;(53). Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=21775962
23. Anderson GG, Palermo JJ, Schilling JD, Roth R, Heuser J, Hultgren SJ. Intracellular bacterial biofilm-like pods in urinary tract infections. *Science* (80-) [Internet]. 2003;301(5629):105–7. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=12843396
24. Hung C-SS, Dodson KW, Hultgren SJ. A murine model of urinary tract infection. *Nat Protoc* [Internet]. 2009/08/01. 2009 Jul;4(8):1230–43. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19644462
25. Bahrani-Mougeot FK, Buckles EL, Lockett C V., Hebel JR, Johnson DE, Tang CM, et al. Type 1 fimbriae and extracellular polysaccharides are preeminent uropathogenic *Escherichia coli* virulence determinants in the murine urinary tract. *Mol Microbiol*. 2002;
26. Thai KH, Thathireddy A, Hsieh MH. Transurethral induction of mouse urinary tract infection. *J Vis Exp* [Internet]. 2010/08/24. 2010 Aug 5;(42):6–9. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=20729806
27. Everts B, Perona-Wright G, Smits HH, Hokke CH, van der Ham AJ, Fitzsimmons CM, et al. Omega-1, a glycoprotein secreted by *Schistosoma mansoni* eggs, drives Th2 responses. *J Exp Med* [Internet]. 2009 Aug;206(8):1673–80. Available from: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2722183&tool=pmcentrez&rendertype=abstract>
28. Schramm G, Hamilton J V, Balog CIA, Wuhrer M, Gronow A, Beckmann S, et al. Molecular characterisation of kappa-5, a major antigenic glycoprotein from *Schistosoma mansoni* eggs. *Mol Biochem Parasitol* [Internet]. 2009 Jul;166(1):4–14. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/19428667>

29. Wang X, Zhou S, Chi Y, Wen X, Hoellwarth J, He L, et al. CD4+CD25+ Treg induction by an HSP60-derived peptide SJMHE1 from *Schistosoma japonicum* is TLR2 dependent. *Eur J Immunol* [Internet]. 2009/11/03. 2009;39(11):3052–65. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=19882655
30. Sun X, Zhou HJ, Lv ZY, Zhang SM, Hu SM, Zheng HQ, et al. [Studies on immunomodulation effect of recombinant Sj16 from *Schistosoma japonicum* on inflammation response of host]. *Zhongguo Ji Sheng Chong Xue Yu Ji Sheng Chong Bing Za Zhi*. 2008;
31. Vennervald BJ, Dunne DW. Morbidity in schistosomiasis: An update. *Current Opinion in Infectious Diseases*. 2004.
32. Li XX, Zhou XN. Co-infection of tuberculosis and parasitic diseases in humans: A systematic review. *Parasites and Vectors*. 2013.
33. Ndyomugenyi R, Kabatereine N. Integrated community-directed treatment for the control of onchocerciasis, schistosomiasis and intestinal helminths infections in Uganda: Advantages and disadvantages. *Trop Med Int Heal*. 2003;
34. Farid Z, Higashi GI, Bassily S, Young SW, Sparks HA. Chronic salmonellosis, urinary schistosomiasis, and massive proteinuria. *Am J Trop Med Hyg* [Internet]. 1972 Sep;21(5):578–81. Available from: <http://www.ncbi.nlm.nih.gov/pubmed/4627631>
35. Tamadonfar KO, Omattage NS, Spaulding CN, Hultgren SJ. Reaching the End of the Line: Urinary Tract Infections. *Microbiol Spectr*. 2019;
36. Yilmaz A, Sevketoglu E, Gedikbasi A, Karyagar S, Kiyak A, Mulazimoglu M, et al. Early prediction of urinary tract infection with urinary neutrophil gelatinase associated lipocalin. *Pediatr Nephrol*. 2009;
37. Zhao J, Wang Z, Chen X, Wang J, Li J. Effects of intravesical liposome-mediated human beta-defensin-2 gene transfection in a mouse urinary tract infection model. *Microbiol Immunol*. 2011;
38. Becknell B, Spencer JD, Carpenter AR, Chen X, Singh A, Ploeger S, et al. Expression and Antimicrobial Function of Beta-Defensin 1 in the Lower Urinary Tract. Neyrolles O, editor. *PLoS One*. 2013 Oct;8(10):e77714.
39. Spencer JD, Jackson AR, Li B, Ching CB, Vonau M, Easterling RS, et al. Expression and Significance of the HIP/PAP and RegIIIy Antimicrobial Peptides during Mammalian Urinary Tract Infection. *PLoS One*. 2015;
40. Danka ES, Hunstad DA. Cathelicidin augments epithelial receptivity and pathogenesis in experimental *Escherichia coli* cystitis. In: *Journal of Infectious Diseases*. 2015.
41. Huang H, Li Y, Liang J, Finkelman FD. Molecular regulation of histamine synthesis. *Frontiers in Immunology*. 2018.
42. Paul WE, Zhu J. How are TH2-type immune responses initiated and amplified? *Nature Reviews Immunology*. 2010.

Figures

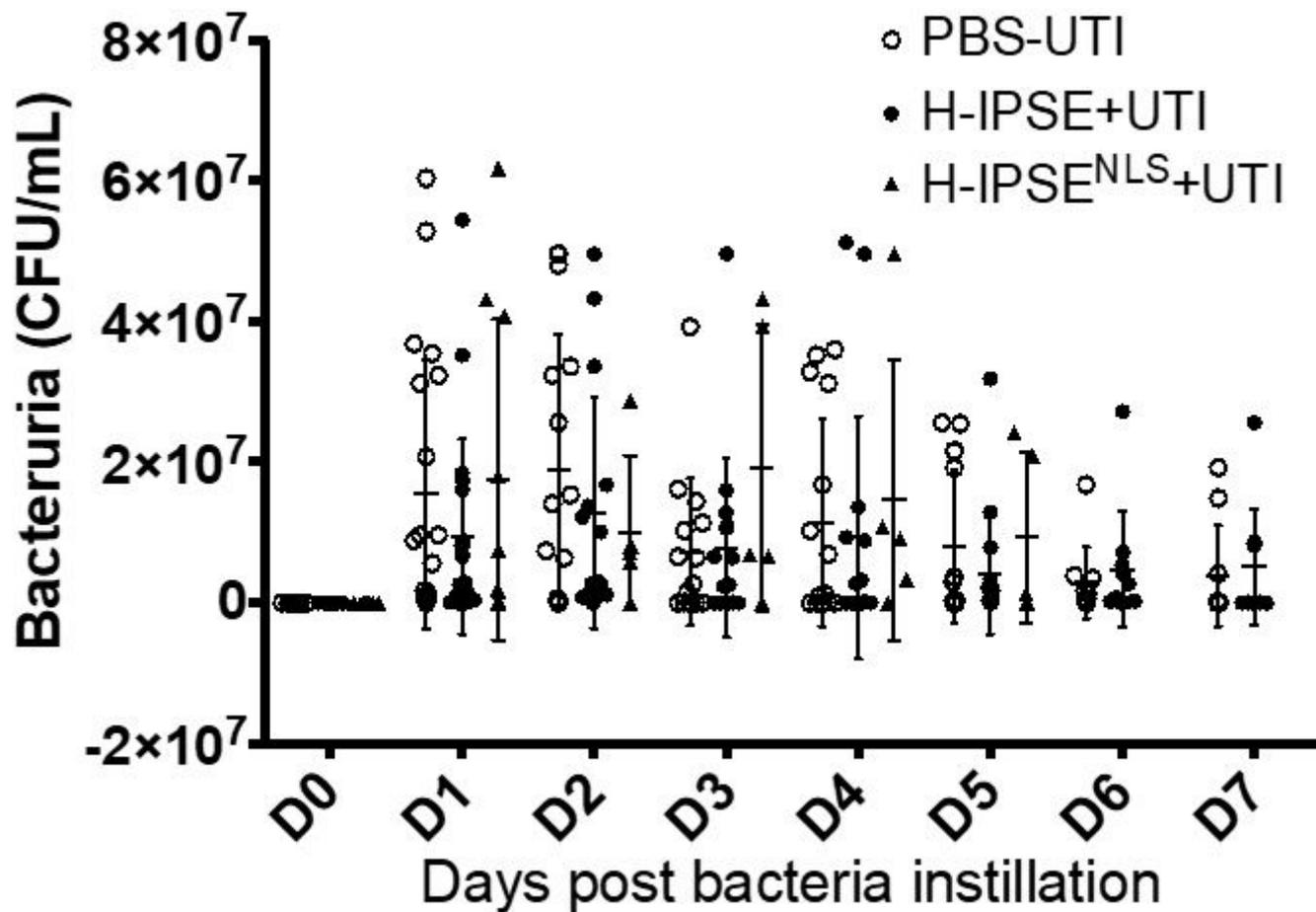


Figure 1

H-IPSE does not affect bacteriuria in mice. “PBS-UTI”, mice given PBS vehicle tail vein injections prior to UTI challenge, “H-IPSE+UTI”, mice administered the H06 ortholog of IPSE before UTI challenge, “H-IPSE^{NLS}+UTI”, mice given a nuclear localization sequence mutant of the H06 ortholog of IPSE prior to UTI challenge. Bars represent mean and standard deviation. Observations were not determined to be statistically significant.

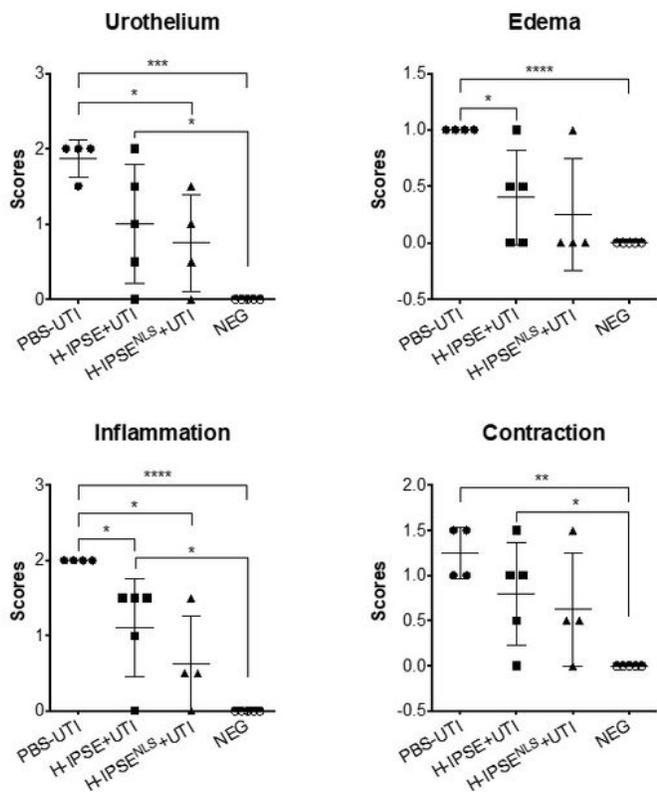
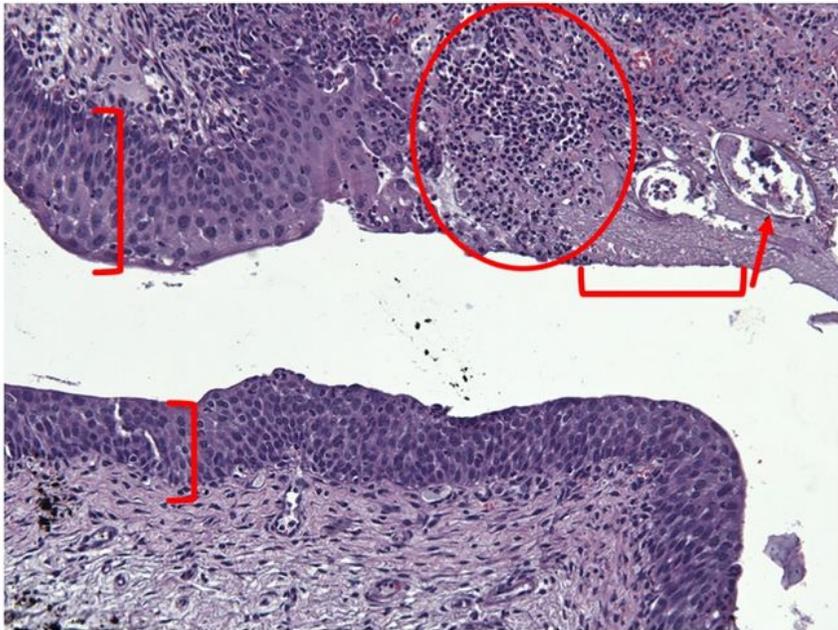


Figure 2

Effects of *S. haematobium* eggs and IPSE on UTI-associated bladder pathogenesis. A) histology demonstrating typical mouse bladder tissue findings during combined *S. haematobium* egg-bacterial co-infection. Many of these features are common to bladders from mice challenged with bacterial uropathogens alone. Noteworthy features include urothelial hyperplasia with reactive changes (vertical brackets), mixed leukocyte infiltrate (subepithelial), ulceration of the urothelium (horizontal bracket)

overlying an egg with degenerated miracidium (arrow), extravasated red blood cells, and mixed acute and chronic inflammatory infiltrate (oval). B) IPSE reduces bladder pathogenesis. “PBS-UTI”, mice given PBS vehicle tail vein injections prior to UTI challenge, “H-IPSE+UTI”, mice administered the H06 ortholog of IPSE before UTI challenge, “H-IPSENLS+UTI”, mice given a nuclear localization sequence mutant of the H06 ortholog of IPSE prior to UTI challenge, “NEG”, unmanipulated mice. Bars represent means and standard deviation. Findings were considered statistically significant at $p < 0.05$. *, **, ***, and **** denotes $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$, respectively.

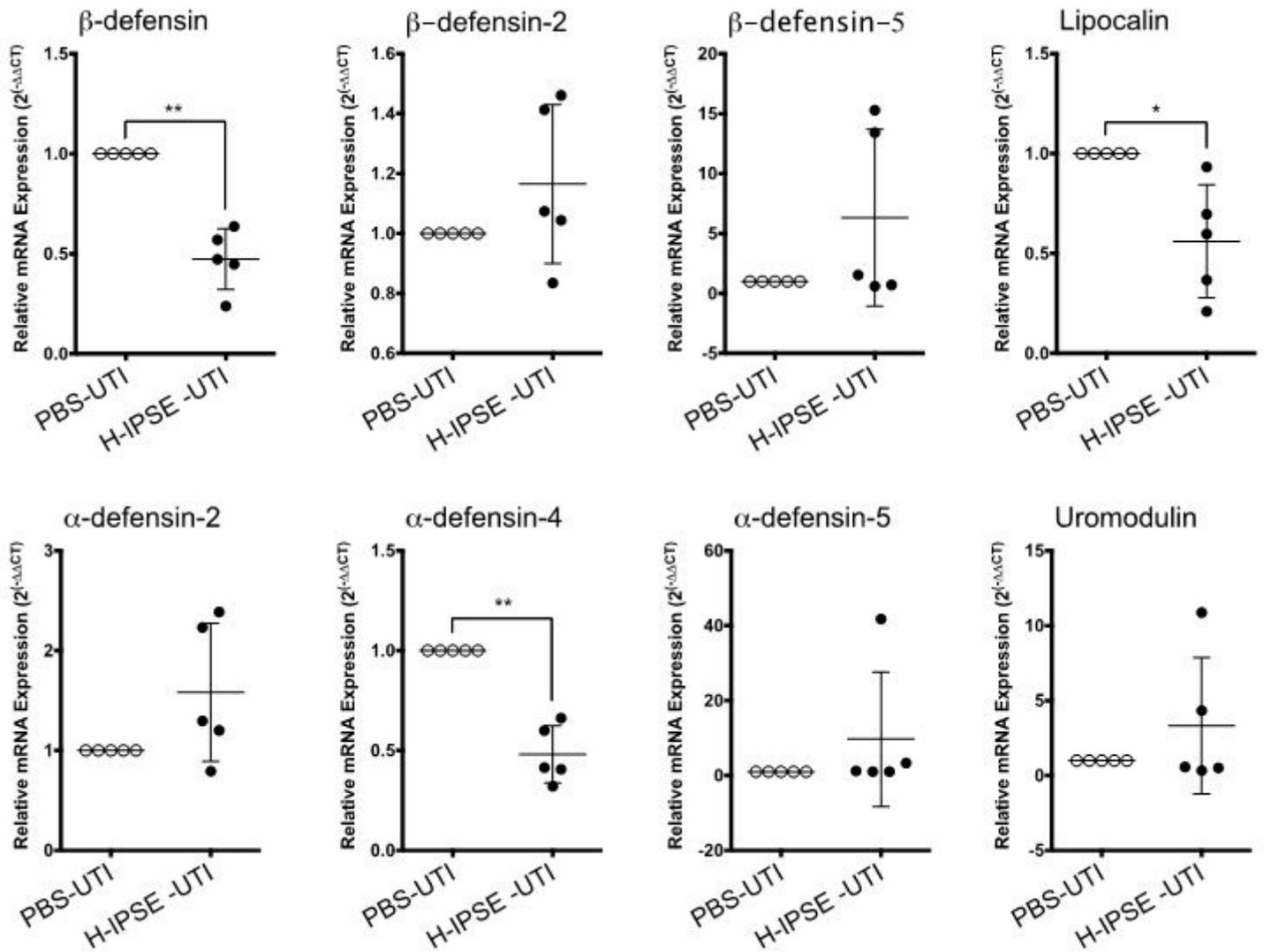


Figure 3

IPSE administration prior to UTI challenge decreases anti-microbial peptide gene expression in the mouse bladder. “PBS-UTI”, mice given PBS vehicle tail vein injections prior to UTI challenge, “H-IPSE+UTI”, mice administered the H06 ortholog of IPSE before UTI challenge. Error bars represent mean and standard deviation. Bars represent mean and standard deviation. Differences between groups were considered statistically significant at $p < 0.05$. * and ** denotes $p < 0.05$ and $p < 0.01$, respectively.

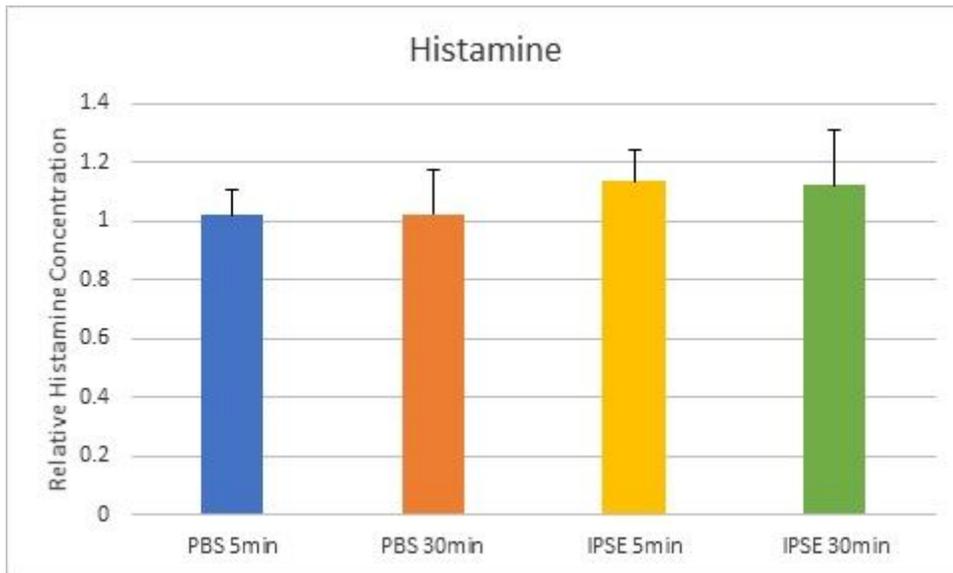


Figure 4

Intravenously administered IPSE Does Not Increase Circulating Histamine Levels. “PBS”, mice injected with phosphate buffered saline, “IPSE”, mice injected with IPSE. Bars represent mean and standard deviation. No significant differences were found in the levels of circulating histamine between PBS- or IPSE-injected groups at either 5-minutes ($p = 0.1473$) or 30-minutes ($p = 0.4374$).

Supplementary Files

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