Supporting Online Material for

Exploitation of the Intestinal Microflora by the Parasitic Nematode *Trichuris muris*

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Materials and Methods

T. muris

Stock infections of *T. muris* were maintained in susceptible mouse strains and adult worms harvested at day 42 p.i. Adults worms were incubated for 4 hours or overnight and eggs collected. Eggs were allowed to embryonate for at least 6 weeks in dH_20 and infectivity established by worm burden in a susceptible mouse strain. Mice were infected with 150-300 embryonated eggs and worm burdens established at day 14 or day 21 p.i. *T. muris* excretory/secretory antigen (E/S) was prepared as follows. Adult worms were cultured in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin and 100 μg/mL streptomycin (all Invitrogen, UK) and E/S antigen was collected after 4 hours incubation. The E/S was pelleted to remove eggs, concentrated using a Centriprep YM-10 (Amicon, Gloucester, UK) and then dialysed against PBS. Protein concentration was determined using a Lowry assay (*S1*).

Animals

AKR and C57BL/6 mice (Harlon Olac, UK) were housed at the University of Manchester. SCID mice were a kind gift from Professor Else. In all experiments, mice were infected when 6-8 weeks old and experimental groups contained four to five animals. All experiments were performed under the regulations of the Home Office Scientific Procedures Act (1986).

Antibiotic Treatment

Animals were treated with 25mg/ml Baytril™ (enrofloxacin) in drinking water.

Cell Culture and Cytokine Analysis

Mesenteric lymph node (MLN) cells were removed from infected mice at day 15 or 18 p.i. The cells were resuspended at a concentration of 5×10^6 cells/mL in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 100 U/mL penicillin and 100 µg/mL streptomycin (all Invitrogen, UK). Cultures were stimulated with 50 µg/mL of *T. muris* E/S at 37°C and 5%CO_2. Cell-free supernatants were harvested after 24 h and stored at –20°C. Cytokine analysis was carried out by Cytokine Bead Assay (BD, UK). The amount of cytokine in cell supernatants was determined by reference to recombinant murine standards.

Antibody Analysis

Analysis of parasite-specific IgG1 and IgG2a production was carried out by capture ELISA. In brief, Immulon IV plates (Dynatech) were coated with *T. muris* E/S (5µg/ml) in carbonate/bicarbonate buffer, pH 9.6, overnight at 4°C. After blocking (3% BSA in PBS, 0.05% Tween), eight serial 2-fold dilutions of sera (from an initial 20-fold dilution) were added to the plates. Parasite-specific antibody was detected using biotinylated rat anti–mouse IgG1 (Serotec Ltd.) and biotinylated rat anti–mouse IgG2a (PharMingen).

Hatching Protocol

For all hatching experiments, eggs were added to bacterial cultures and incubated for 2 hours at 37ºC, 5%$C0_2$ or with sections of mouse colon overnight at 37ºC, 5%$C0_2$. For mannose treatment, 50mM or 100mM mannose was added to cultures 30mins before assay and left in cultures for the duration of the assay.

Bacteria

All bacterial strains used in the study were routinely grown in LB broth overnight at 37ºC and shaking at 200rpm. Strains AAEC072A (KO), PK116 (WT) and plasmid pLB254 (KI) were a generous gift of Prof. P. Klemm, Technical University of Denmark, Lyngby, Denmark.

Yeast

Saccharomyces cerevisiae cultures were a kind gift from Dr Peter Reid (University of Manchester). Cultures were grown overnight at 37ºC, shaking at 200rpm in YPD media (Sigma, UK).

Purification of FimH.

The his-tagged mannose binding domain of FimH was purified from strain DH5α (pPKL241) as described previously (*S2*).

Far Western

T. muris eggs were boiled for 10mins in reducing buffer (13% 1.5M DDT, 12.5% 0.5M Tris, 4.0% SDS, 20% glycerol) and resolved by gel electrophoresis on a 4-12% gradient NuPAGE gel run in NuPage MES SDS running buffer (Invitrogen, UK). After transfer to nitrocellulose in NuPage Transfer buffer (Invitrogen, UK), the blots were incubated in 5% marvel powder for 1.5hr, washed in TBS Tween20 (TBST) and probed with 6µg/ml purified FimH overnight at 4ºC. After washing with TBST, blots were probed with mouse anti-his antibody used at 1:1000 (Qiagen) and then peroxidise conjugated anti-

mouse IgG (Sigma, UK) used at 1:3000. The membrane was incubated with a chemiluminescence reagent kit (PerkinElmer Life Sciences, Boston, USA) and FimH binding detected by exposure of the membranes to Kodak film in an autoradiography cassette for 1min.

Electron Microscopy

The fimbriae of *E. coli* strains were examined with a transmission electron microscope. Formvar-coated copper grids (600 mesh, Agar Scientific) were carbon coated (Bio-Rad E6200 Carbon Coater) and plasma glowed (Plasma Barrel Etcher PT7150; Fisons) prior to use. Bacterial cells from an 18hr aerobic culture were collected by centrifugation (6000g for 3mins), washed and resuspended in phosphate-buffered saline (PBS, pH 7.4). 10μL of the cell suspension was added to the grids and the bacteria were stained with 2% (wt/vol) methylamine tungstate (pH 6.5). All bacteria were photographed using an FEI Tecnai 12 electron microscope (FEI Company, Eindhoven, The Netherlands) at 100kV.

Peptide Extraction and Mass Spectrometry

Silver stained bands of interest in agarose gel were washed in Acetonitrile for 5min and dried in a vacuum centrifuge for 5min. Samples were then reduced in 10mM DTT in 25mM NH_4HCO_3 for 1 hr at 56ºC. Following alkylation in 55mM Iodoacetamide in 25mM NH_4HCO_3 for 45mins at room temperature, samples were washed twice in acetonitrile and dried in a vacuum centrifuge. Samples were then trypsinised overnight in 62.5ng trypsin and 25mM NH_4CO_3 at 37ºC. Peptides were solubilised in 20mM NH_4HCO_3 for 20mins, then twice in 5% formic acid in 50% Acetonitrile for 20mins.

Trypsinized samples were analyzed using an Ultimate 3000 LC system (LC-Packings) coupled to a HCT Ultra ion Trap mass spectrometer (Bruker Daltonics). 5µl of sample was concentrated/desalted on a pre-column (5 mm x 300 µm i.d., LC-Packings). The peptides were then separated using a gradient from 98% A (0.1% formic acid in water) and 1% B (0.1% formic acid in acetonitrile) to 75% A and 25% B, over 40mins at 300 nl min-1, using a C18 PepMap column (150 mm x 75 µm i.d., LCPackings). Data acquired were analyzed using SWISS-PROT and NEMBASE databases using the ProteinLynx global server 1.1 software (Waters).

Video and Images

Images were acquired on an AS MDW live cell imaging system (Leica) using a 63x objective, the BGR filter set (Chroma 61002) and a (green (GFP)) Precise LED fluorescent light source. Cells were maintained at 37ºC and 5%CO_2. The images were collected using a Coolsnap HQ (Photometrics) camera.

Statistics

Significant differences ($p<0.05$) between experimental group worm burdens were determined using the Mann-Whitney U test. Significant differences ($p<0.05$) between experimental groups for other parameters was determined using ANOVA.

Supplemental Figures

Movie S1

Video of *T. muris* hatching *in vitro* in *E. coli* bacterial suspension. Eggs were incubated in *E.coli* for 1hr then time lapse photos taken over 30mins at 2min intervals on a Leica AS MDW.

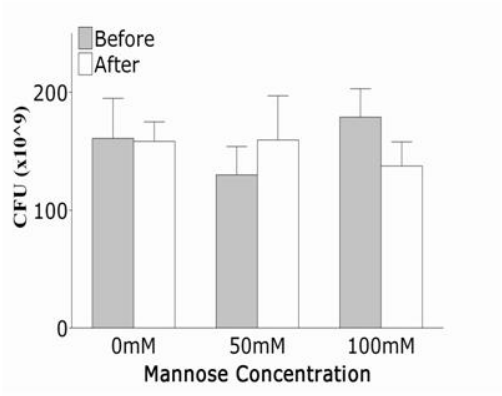

Fig S1

Viability of bacteria before and after hatching assay. *E. coli* was treated with 0mM, 50mM or 100mM Mannose for 30mins before eggs were added and then left for 2 hours at 37°C. Bacterial counts were performed on cultures before mannose was added and cultures after the assay using LB plates in duplicate. Plates were incubated at 37ºC overnight and the number of colonies counted. All figures represented as mean ± SEM.

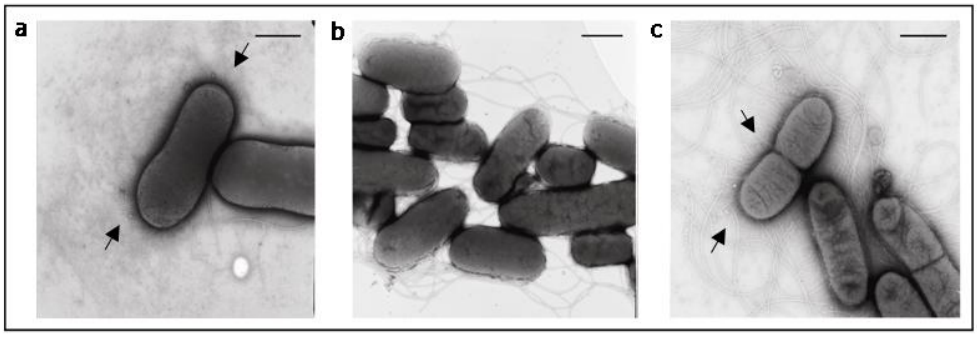

Fig S2

Electron micrograph of (a)Wild type *E. coli*, (b) FimKO and(c) FimKI *E.coli*. Type 1 fimbriae indicated by arrows. Cells were negatively stained with 0.5% ammonium molybdate, pH7.2. Bar, 1μm.

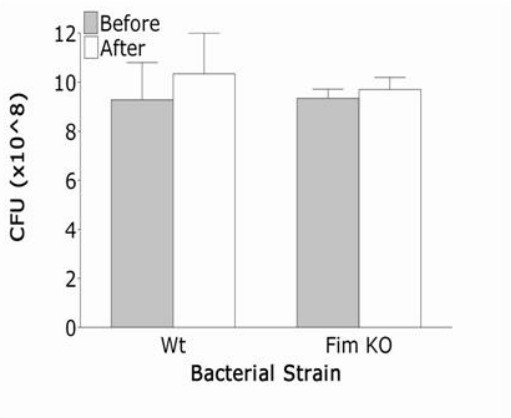

Fig S3

Viability of Wild type *E.coli* and Fim KO *E.coli* before and after hatching assay. Suspensions of *E. coli* and FimKO *E.coli* were treated with 0mM or 50mM mannose for 30mins before eggs were added and left for 2 hours at 37°C. Bacterial counts were performed on LB plates in duplicate. Plates were incubated at 37ºC overnight and the number of colonies counted. All figures represented as mean ± SEM.

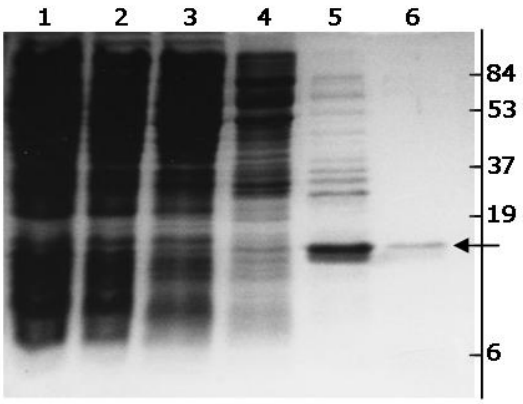

Fig S4

Purified his-tagged mannose binding domain of FimH. Lane 1, total lysate; Lane 2 Column flow through; Lane 3 Column wash using 10 mM imidazole; Lane 4 Column wash using 50 mM imidazole; Lanes 5 and 6 Column wash was using 250 mM imidazole. The arrow denotes the purified his-tagged FimH trunckate in lane 6. The numbers on the right hand side denote molecular weight markers in kDa.

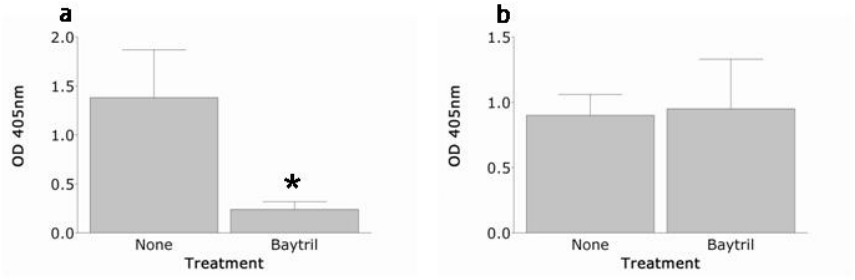

Fig S5

Serum parasite specific IgG1 (a) and IgG2a (b) from infected C57BL/6 mice treated with enrofloxacin from day -2 to day 17 p.i. or from untreated control mice. All figures represented as mean ± SEM. *$p<0.01$ using ANOVA.

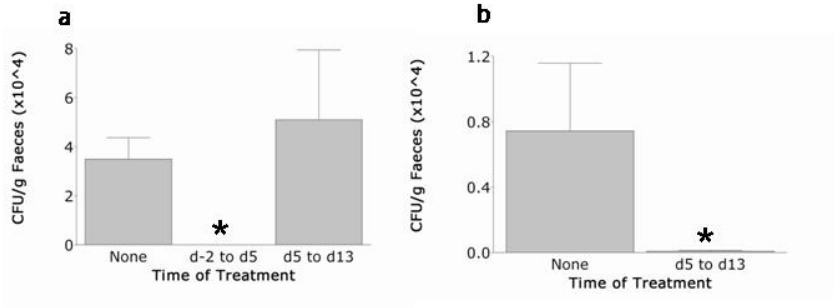

Fig S6

Bacterial counts from enrofloxacin treated mice at the beginning or end only of infection measured at (a) d0 or (b) d14 p.i. Bacterial counts of faeces were performed on LB plates in duplicate. Plates were incubated at 37ºC overnight and the number of colonies counted. All figures represented as mean ± SEM. *$p<0.05$ using ANOVA

References

S1. Lowry, O. H., Rosebrough, N. J., Farr, A. L., Randall, R. J., Protein Measurement with the Folin Phenol Reagent *JBC* **193**,265-275 (1951)

S2. Schembri, M. A., Hasman, H., Klemm, P., Expression and purification of the mannose recognition domain of the FimH adhesin *FEMS Microbiol Lett* **188**,147-151 (2000)