METHODS

Mice and bacteria. MyD88$^{-/-}$ mice were provided by S. Akira. C57BL/6, C57BL/6 Ly5.1 and C3H/HeJ mice were purchased from Jackson Laboratory. All mice were kept and bred at Memorial Sloan-Kettering Cancer Center Research Animal Resource Center. All animal procedures were approved and performed according to institutional guidelines for animal care. All experiments were performed with the VRE from ATCC (stock number 700221), except for 3 experiment 1a, which was done with a clinical VRE isolate from the microbiology laboratory at MSKCC.

Generation of bone marrow chimaeric mice. Recipient wild-type (CD45.1) or MyD88-deficient (CD45.2) mice were lethally irradiated with 950 cGy using a ^{137}Cs source and injected intravenously 2–3 h later with 5×10^6 bone marrow cells derived from the tibias and femurs of the respective donors. bone marrow chimaeric mice were used 7 weeks after engrafting.

Real-time PCR analysis. The small intestine was divided into four parts; a 1.5 cm segment was excised from the distal portion and total RNA was isolated using the Trizol reagent (Invitrogen). DNase-treated RNA underwent randomly primed cDNA synthesis and real-time PCR analysis. SYBR Green-based real-time PCR was performed using the DyNAmo SYBR Green qPCR Kit (Finnzymes). RegIIIγ- and angiogenin-4-specific primers were obtained from Qiagen. General cryptdin primer was used as described previously[27]. Signals were normalized to *Gapdh* 4 RNA (forward: 5′-ACCACAGTCCATGCCATCAC-3′; reverse: 5′-TCCACCACCCTGTTGCTGTA-3′). Normalized data were used to quantify relative levels of RegIIIγ using ΔΔCt analysis.

Western blot analysis. Small intestines were divided into four pieces; samples were taken from the most distal part and western blotting using a polyclonal RegIIIγ antiserum or an anti-tubulin antibody (Santa Cruz) as loading control was performed as described previously[10].

Western blot signals were analysed by densitometric measurements and subsequently quantified using the NIH-Image software program. Values obtained for RegIIIγ immunoblots were normalized to the optical density of corresponding immunoblots for tubulin.

Immunohistochemistry. Freshly isolated small intestine was divided into four parts. The fourth distal part was fixed in formalin and embedded in paraffin. Immunohistochemical staining for RegIIIγ was performed using the DAKO Envision System for rabbit primary antibodies according to the manufacturer's protocol. RegIIIγ polyclonal antiserum[10] was diluted 1:500 in PBS containing 1% BSA. Control slides were stained with pre-immune serum or rabbit immunoglobulin G instead of the primary antibody and did not show any positive staining.

***In vivo* luminal killing.** After anaesthesia a midline laparotomy incision was made. The intestine was occluded with a vascular clip 1 cm proximal to the ileo–caecal junction and 3 cm proximal to this point. Care was taken to avoid disrupting the mesenteric vascular arcades. The length of intestine between the two clips was injected with 250 μl PBS containing 1,000 VRE. After 2 h, mice were killed by exposure to carbon dioxide and the luminal fluid of the isolated segment was harvested and plated on Enterococcose I agar plates. For RegIIIγ-blocking experiments, 250 μl of a 1:10 dilution of RegIIIγ polyclonal antiserum[10] or pre-immune serum in PBS was injected into ileal loops of wild-type and antibiotic-treated mice. After 20 min, 250 μl PBS containing 1,000 VRE was injected. For reconstitution experiments, 250 μl MES-Buffer (25 mM, pH 6, 25 mM NaCl) containing 20 μM purified recombinant RegIIIγ was injected before adding 1,000 VRE in 100 μl MES-Buffer into the ileal loop. RegIIIγ was purified as described previously[10]. As a control, purified β2-microglobulin was substituted for RegIIIγ. After 2 h the animals were killed by exposure to carbon dioxide immediately before harvesting the isolated section of the small intestine.

Determination of the vancomycin levels. The colon was dissociated in 1 ml PBS and centrifuged for 10 min at 13,000*g*. Vancomycin levels were analytically determined by a particle-enhanced turbidimetric inhibition immunoassay method[28].

Array analysis and ELISA. Array analysis (Toll-like receptor signalling pathway PCR array from Superarray) was performed to determine the bioactivity of LPS and LTA in the specific concentrations used for the experiments (unpublished results). For intraperitoneal administration of LPS, mice were injected with 1 μg LPS. After 2 h, serum TNF levels were determined by ELISA (OptEIA kit from BD Pharmingen).

Statistical analysis. Statistical analysis was performed on Prism software. All *P*-values < 0.05 were considered to be significant. Error bars denote s.e.m.

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28. Hammett-Stabler, C. A. & Johns, T. Laboratory guidelines for monitoring of antimicrobial drugs. *Clin. Chem.* **44,** 1129–1140 (1998).