ONLINE METHODS

Fish and parasite infection. Rainbow trout (Limestone Springs Fish Farm) were maintained in the laboratory of J.O.S. as described[39]. For sublethal infection with *C. shasta,* groups of rainbow trout (20–30 g; Roaring River Hatchery) were held in cages in the Klamath river at the Keno Eddy field site, where the parasite density is less than five parasites per liter of river water[40]. After 3 d of exposure, fish were then transferred to the John L. Fryer Salmon Disease Laboratory (Oregon State University) and were raised at 18 °C as described[40]. As a control, groups of fish were held in the laboratory without exposure to infectious water of the Klamath River. After 3 months, surviving fish were sampled as described below. Our infection protocol yields an infection rate of 89% ± 18% (s.d.) for exposed fish. Typically, 12% ± 2.4% (s.d.) of the infected fish die during the infection phase (data obtained from a total of 420 fish in ten independent studies). Most fish that succumb to the parasite die within the first 1–2 months. Very rarely fish die after 3 months after infection; at 3 months, living fish are considered survivors. Animal procedures were approved by the Institutional Animal Care and Use Committees of the University of Pennsylvania and Oregon State University, respectively.

Purification of IgT and IgM. Affinity columns were prepared by coupling of mouse mAb to trout IgT (38.5; isotype, IgG2b) or to trout IgM (1.14; isotype, IgG1) to *N*-hydroxysuccinimide-activated Sepharose according to the manufacturer's instructions (GE Healthcare). Anti–trout IgM was a gift from G. Warr. For isolation of native trout IgM and IgT, a serum sample pooled from several individual trout was diluted 1:2 in PBS-EDTA (1× PBS containing 20 mM EDTA, pH 7.2) and was applied to the anti–trout IgM column equilibrated in PBS-EDTA. Thereafter, the flow-through was applied to the anti–trout IgT column. After several washes of both affinity columns with PBS-EDTA, bound IgM and IgT were eluted with 0.1 M glycine, pH 2.5, and were immediately neutralized with 1 M Tris, pH 9.0. The eluted IgM and IgT fractions were concentrated and subjected to one additional purification step of gel-filtration chromatography with a Superdex 200 fast-performance liquid chromatography column (GE Healthcare) equilibrated in PBS-EDTA.

Immunofluorescence microscopy studies. Cryosections of tissue samples were fixed for 3 min in 10% (vol/vol) neutral buffered formalin. Background autofluorescence was eliminated by treatment of cryosections for 10 min with 0.1 M glycine, pH 2.3. For detection of IgT^+ and IgM^+ cells in all tissues and of *C. shasta* in the intestine, cryosections were incubated overnight at 4 °C with rabbit anti–trout IgT (0.2 μg/ml), mouse anti–trout IgM (IgG1 isotype; 1 μg/ml) and/or mouse anti–*C. shasta* (D8; IgG2a isotype; 1 μg/ml). After being washed three times with 1× PBS containing 0.2% (vol/vol) Triton X-100 and 0.5% (wt/vol) BSA, pH 7.2, samples were incubated for 2 h at 20 °C with indocarbocyanine-conjugated donkey anti–rabbit IgG (711-165-152), indodicarbocyanine-conjugated goat anti–mouse IgG1 (115-175-205) and/or carbocyanine-conjugated goat anti-mouse IgG2a (115-225-206; 2.5 μg/ml each; all from Jackson Scientific). For visualization of coating of gut luminal bacteria with IgM and IgT, gut bacteria (100 μl; absorbance at 600 nm, 0.3) were incubated for 2 h at 20 °C with anti–trout IgM or anti–trout IgT (1 μg/ml each) or their respective isotype-matched control antibodies. After samples were washed as described above, the secondary antibodies indodicarbocyanine-conjugated goat anti–mouse IgG1 or indocarbocyanine-conjugated donkey anti–rabbit IgG (1 μg/ml) were added. After incubation for 1 h at 4 °C, bacteria where washed as described above. Before being mounted, tissue samples were stained with DAPI (4,6-diamidino-2-phenylindole; 1 μg/ml; Invitrogen) and bacteria were stained with a mixed solution of DAPI and Hoechst 33342 dye (5 μg/ml each; Molecular Probes). Images were acquired and analyzed with a Nikon E600 fluorescence microscope and IPLab imaging software (BD Biosciences).

ELISA. The fluid phase of a parasite lysate (from *C. shasta*) was used as the antigen for ELISA. Parasites were obtained from severely infected fish at early stages of infection (4–8 d after exposure). After collection, parasites were washed three times and resuspended in PBS, pH 7.2. Lysates of *C. shasta* were prepared by three cycles of freezing and thawing, followed by centrifugation at 15,000*g* for 20 min. Supernatants of lysates were used as the antigen for measurement of titers of specific anti-parasite IgT and IgM by ELISA. Antigen (20 μg/ml in PBS, pH 7.2; 50 μl per well) was absorbed overnight at 4 °C onto Maxisorp microplates (Thermo Fisher Scientific). Nonspecific binding sites were then blocked with 8% (wt/vol) skim milk in PBS, pH 7.2. Plates were washed with 1× PBS containing 10 mM EDTA and 0.05% (vol/vol) Tween 20, pH 7.2. Gut mucus or serum samples from control or surviving fish were serially diluted in 1× PBS containing 10 mM EDTA, pH 7.2. For analysis of the binding of IgT or IgM to parasite antigens, biotin-labeled anti–trout IgM or anti–trout IgT (2 μg/ml in 1× PBS containing 10 mM EDTA and 1% (wt/vol) BSA, pH 7.2) was added to each well. After 2 h of incubation at 4 °C, plates were washed and bound antibodies were detected by incubation with Pierce High Sensitivity Streptavidin-HRP (horseradish peroxidase; 0.25 μg/ml; Thermo Fisher Scientific), followed by color development with ABTS (2,2′-azino-di-(3 ethylbenzthiazoline sulfonic acid); Invitrogen) as a substrate. After 30–45 min, the color reaction was stopped with a solution of 1% (wt/vol) SDS and absorbance was measured at 405 nm. *C. shasta*–specific antibody titers are presented as the reciprocal of the highest serum or gut mucus dilution that provided an average absorbance exceeding twofold the average background absorbance at 405 nm. Before being added to ELISA plates, gut mucus and serum samples were preincubated with a molar excess of anti–trout IgM (for IgT titers) or anti–trout IgT (for IgM titers) to avoid competition of IgM or IgT binding, respectively.

Statistical analysis. An unpaired Student's *t*-test (Excel version 11.0; Microsoft) and nonparametric Mann–Whitney *t*-test (Prism version 5.0; GraphPad) were used for analysis of differences between two groups. *P* values of 0.05 or less were considered statistically significant.

Additional methods. Information on the collection of serum, gut mucus, intestinal bacteria and leukocytes; flow cytometry and morphological, gene-expression and gel-filtration analyses; SDS-PAGE and immunoblot analysis; phagocytosis and intracellular bacterial killing assays; B cell proliferation assay and stimulation of IgT and IgM production by microbial products; cloning, sequence analyses and expression of trout pIgR in MDCK cells; and coimmunoprecipitation is available in the **Supplementary Methods**.

39. Zhang, Y.A. *et al.* Conservation of structural and functional features in a primordial CD80/86 molecule from rainbow trout (*Oncorhynchus mykiss*), a primitive teleost fish. *J. Immunol.* **183**, 83–96 (2009).
40. Stocking, R.W., Holt, R.A., Foot, J.S. & Bartholomew, J.L. Spatial and temporal occurrence of the salmonid parasite *Ceratomyxa shasta* in the Oregon-California Klamath river basin. *J. Aquat. Anim. Health* **18**, 194–202 (2006).

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