SUPPLEMENTAL TABLES

Table S1, related to Table 1. Odds Ratios for Association of Heterozygosity at *LTA4H* SNPs with Susceptibility to TB Stratified by Age.

SNP	All ages		All ages adjusted for sex		age<35 n=351	age 35-50 n=199	age>50 n=139
	OR[a]	*P*	OR[a]	*P*	OR[a] (95% CI)	OR[a] (95% CI)	OR[a] (95% CI)
rs1978331 T/C	0.71	0.002	0.67	0.002	0.75 (0.57-0.98)	0.66 (0.47-0.92)	0.73 (0.49-1.08)
rs17677715 T/C	0.69	0.478	0.56	0.316	1.14 (0.30-3.68)	0.40 (0.01-2.86)	na[b]
rs2247570 A/G	0.66	0.018	0.58	0.007	0.55 (0.34-0.88)	0.81 (0.47-1.36)	0.77 (0.39-1.40)
rs2660898 T/G	0.64	0.00004	0.64	0.001	0.69 (0.53-0.90)	0.67 (0.48-0.94)	0.51 (0.34-0.77)
rs2660845 G/A	0.84	0.099	0.67	0.002	0.80 (0.61-1.06)	0.83 (0.59-1.16)	0.95 (0.64-1.39)
rs2540475 C/T	0.82	0.068	0.85	0.219	0.80 (0.61-1.06)	0.83 (0.59-1.16)	0.86 (0.57-1.27)

a) ORs for each SNP are calculated for heterozygotes versus homozygotes in cases versus controls.
b) At rs17677715, fewer than five individuals age >50 carried the C allele.

Table S2, related to Table 1. Associations of TB and Leprosy with Heterozygosity at rs1978331 and rs2660898.

Disease	Group	rs1978331				rs2660898				rs1978331 and rs2660898			
		TC	TT+CC	OR[a]	*P*	TG	TT+GG	OR[a]	*P*	not both heterozygous[b]	both heterozygous[c]	OR[a]	*P*
Tuberculosis	All TB	261	396	0.71	0.002	263	395	0.64	0.00004	476	156	0.65	0.0003
	Pulmonary TB	145	224	0.70	0.006	153	224	0.66	0.001	272	87	0.63	0.002
	Meningeal TB	116	172	0.73	0.026	110	171	0.62	0.001	204	69	0.67	0.012
	Controls	359	389			382	369			492	248		
Leprosy	Multibacillary without ENL	190	247	0.62	0.001	146	247	0.70	0.021	273	116	0.68	0.016
	Multibacillary with ENL	59	61	0.78	0.235	57	56	1.21	0.380	67	45	1.08	0.742
	Paucibacillary	182	146			147	175			194	121		

a) ORs for each SNP are calculated for heterozygotes versus homozygotes in cases versus controls.
b) Individuals homozygous for one or both SNPs rs1978331 or rs2660898
c) Individuals heterozygous for both SNPs rs1978331 and rs2660898

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Animal Care and Strains

Adult fish were maintained as described (Cosma et al., 2006). Wild-type AB zebrafish larvae were maintained and infected with bacteria by injection into the caudal vein or hindbrain ventricle as described (Clay et al., 2007; Clay et al., 2008; Cosma et al., 2006). Mutants were backcrossed (at least twice per mutant line) to wildtype ABs and carriers were identified based on sibling incrosses that yielded approximately 25% mutants.

Bacterial Strains and Enumeration

All bacterial strains are based on the wild-type *M. marinum* strain M (ATCC #BAA-535) that were rendered red or green fluorescent (Cosma et al., 2006). *erp* mutant bacteria were derived as described (Clay et al., 2008). Bacterial counts were determined as described by plating (Clay et al., 2008). Bacterial burden was also assessed by fluorescence pixel counts (FPC). After images have been taken with standardized exposure times, FPC integrates the number of pixels in each image with values above a background threshold, as determined by matched images of uninfected animals.

Mutagenesis and Screening

Early pressure gynogenetic diploids were generated as described (Johnson et al., 1995) and were infected by caudal vein at 48 hours post-fertilization (hpf) with 150-200 GFP-expressing wildtype *M. marinum* (Clay et al., 2007). Each clutch was split into two groups of 15-30 animals that were infected using different needles. Only clutches that

displayed a mutant phenotype in both groups were pursued for further analysis.

Mapping and Sequencing

Putative mutants were outcrossed to the wildtype WIK strain and mutants and carriers identified by random crosses between siblings. Mutant bulk segregant analysis was performed on mutant progeny and phenotypically wildtype. Animals collected from these incrosses were euthanized and lysed as described (Bahary et al., 2004). Exons were identified based on consensus splice sites as well as sequence homology and exon/intron conservation with the human *lta4h* gene as described (Bahary et al., 2004). After exhausting existing markers, new SNP markers were developed including two SNPs in a 3' intron of ENSDARG00000016381, a relative of the human PRKWNK1 gene. After amplification with the primers WNK-SNP1F (5'CGACCCCCAAAATAACAATG3') and WNK-SNP1R (5' CTCCATGCAGTAGCCATGAA 3'), these SNPs create two *NcoI* restriction site polymorphisms, resulting in a 0.4 kb and 0.6 fragment on WIK chromosomes and a 0.25 kb and 0.75 kb fragment on an AB chromosome. A critical interval was determined based on the Ensembl Zv6 assembly (www.ensembl.org).

Exons were identified based on consensus splice sites as well as sequence homology and exon/intron conservation with the human *lta4h* gene. All 19 exons and their 5' and 3' exon/intron boundaries were sequenced on both strands. Predicted cDNA was confirmed after reverse transcription of RNA extracted from six dpf uninfected embryos as described (Clay et al., 2007) with Accuscript HF (Stratagene).

Quantitative Real-time PCR (qRT-PCR)

qRT-PCR was performed on RNA extracted from pools of 20-30 larvae at indicated time points as described (Clay et al., 2007). Each assessment consisted of mean values of three independent pools. *tnf* mRNA levels were assessed as described (Clay et al., 2007). *lta4h* mRNA levels were assessed using SYBR green and a primer pair that spanned the first intron: 5' TGTGGAGGTCCTGGAGGATA 3' and
5' TCAAAAGGCAGGGTGATTTC 3'.

Retroviral Insertion Mutant

The retroviral insertion mutant *zm5961* was identified from a sperm library maintained by Znomics (Portland, OR). It contains a retroviral insertion within the seventh exon of the *lta4h* gene. This strain was crossed twice to wildtype AB animals and the insertion was followed using the viral specific primer
5' ACAGACACAGATAAGTTGCTGGCC 3' and the primer 5961.2
(5' CACAATCTCTTCATGCATCTTGG 3') which lies within the seventh intron of the *lta4h* gene and is adjacent to the retroviral insertion. Homozygotes were confirmed by splitting animal lysates and confirming the presence of the viral insertion band and the absence of a wildtype band using primers on either side of the insertion: lt4hx78f
(5' CATGGCCACCAGTATGTCAG 3') and lt4hx78r
(5' CCTAAAAACACCCGTGCAGT 3').

Microscopy

Images from Figures 1E and 1F were taken on a Nikon SMZ1500 microscope equipped with an X-Cite 120 Fluorescence Illumination System (EXFO). Images were acquired using a DS-2Mv digital camera and a Digital Sight DS-L1 system (Nikon). All other microscopy was performed on a Nikon E600 equipped with DIC optics, a Nikon D-FL-E fluorescence unit with 100 W mercury lamp and MFC-1000 z-step controller (Applied Scientific Instrumentation). Objectives used included 10x Plan Fluor, 0.3 NA, 20x Plan Fluor, 0.5 NA, 40x Plan Fluor, 0.75 NA, and 60x water Fluor, 1.0 NA. Wide-field fluorescence and DIC images were captured on a CoolSnap HQ CCD camera (Photometrics) with MetaMorph 7.1 (Molecular Devices)

lta4h Morpholinos

Morpholinos were obtained from Genetools (Eugene, OR) and injected at the one- to four-cell stage. Control and TNF receptor morpholinos were used as described (Clay et al., 2008). The *lta4h* morpholino

5’ AGCTAGGGTCTGAAACTGGAGTCAT 3’ was injected at 10-60 µM. All morpholino infections were performed on paired needles; an equal number of morpholino and control animals were infected on each needle in an alternating sequence to reduce variations in dosage between groups.

In situ Hybridization

Fluorescent *in situ* hybridization was performed as described (Clay et al., 2007; Clay et al., 2008). For *lta4h*, an *lta4h* cDNA was generated from AB wildtype animals using Accuscript HF (Stratagene). A 1.4 kb cDNA fragment was generated using the primers 5’

TGTGGAGGTCCTGGAGGATA 3' and 5' TGATCCACGGATCTCCTTAAA 3'. This fragment was subcloned into the pSC-A vector (Stratagene). M13F and M13R primers were then used in standard PCR to generate template for *in vitro* transcription reactions using T3 RNA polymerase for antisense probe. *tnf* and *fms* probes were generated as described (Clay et al., 2007; Clay et al., 2008). For *in situ* hybridization of clutches with mixed genotypes, genotyping took place after documentation of staining. Larvae were lysed as described and genotyped for relevant markers (Bahary et al., 2004).

Antibody and Annexin V Staining

Antibody staining for MPO and iNOS was performed as described (Clay et al., 2007; Clay et al., 2008). For Annexin V staining, a 1/10 dilution of Annexin V-AlexaFluor 488 (Invitrogen) was microinjected into the caudal vein of 4 dpi animals and quantitation was performed four hours later.

Neutral Red and Sudan Black Staining

Neutral red and Sudan black staining was performed as described (Herbomel et al., 2001; Le Guyader et al., 2008).

Leukotriene B_4 and Lipoxin Injections

Approximately 10 nL of Leukotriene B_4 (Cayman Chemical), Lipoxin A_4 (Calbiochem) or 15-epi Lipoxin A_4 (Calbiochem) was microinjected at the concentrations indicated into the hindbrain, caudal vein or right ear as described (Cosma et al., 2006; Le Guyader et al., 2008) and detailed in the main text. Each eicosanoid was injected in 1% ethanol/PBS,

which also served as the vehicle control. Leukotriene B_4 was injected with 1% phenol red to aid visualization of the injection process, while Lipoxin A_4 was injected in the absence of phenol red. To assess, the effects of lipoxins on neutrophil migration, three dpf animals were pre-injected in the caudal vein with either 1% ethanol in PBS or $3.5x10^{-14}$ mol of LXA_4 in 1% ethanol/PBS (right). Six hours after this initial injection, 1% ethanol/PBS or $1.5x10^{-14}$ mol LTB_4 in 1% ethanol/PBS was injected into the right ear. At four hours after the ear injection, fish were fixed and stained with Sudan Black to identify neutrophils that had migrated to the ear.

Eicosanoid Pathway Inhibitors

Small molecule inhibitors of enzymes required for the biosynthesis of leukotrienes or lipoxins were administered by soaking. All administration began at 2 dpf. Bestatin (Cayman Chemical) or vehicle alone was administered at a concentration of 50 µM in 1% DMSO overnight prior to the ear injections of LTB_4. For infection experiments, 100 µM bestatin in 0.25% DMSO or vehicle alone was administered for 5 dpi, with water changed daily. U75302 (BIOMOL) or vehicle alone (0.5% DMSO) was administered at a concentration of 3 µM overnight for ear injection and infection experiments. PD-146176 (BIOMOL) was administered at 1µM overnight and ear injections were performed at 3 dpf. All ear injections were performed into the right ear using 1% ethanol/PBS as a vehicle control or $1.5x10^{-14}$ mol LTB_4 in 1% ethanol/PBS. At four hours after the ear injection, fish were fixed and stained with Sudan Black to identify neutrophils that had migrated to the ear.

Clinical Studies

A case-control study design was used to examine associations of *LTA4H* polymorphisms with susceptibility to and mortality from TB and with susceptibility to leprosy. TB cases (N=692) (pulmonary or meningeal, >14 years old, HIV-negative) were enrolled in Vietnam as previously described (Hawn et al., 2006; Thuong et al., 2007). Briefly, pulmonary TB cases had clinical symptoms and chest X-ray results consistent with active TB, confirmation of *M. tuberculosis* cultured from sputum, and no evidence of extrapulmonary or miliary disease. Meningeal TB cases had clinical evidence of meningitis (defined as nuchal rigidity with abnormal cerebrospinal fluid (CSF)) and (i) microbiologic confirmation of acid-fast bacilli stain of, or *M. tuberculosis* culture from the CSF (N=225) or (ii) evidence of pulmonary TB as above (N=74). Survival data up to approximately nine months post enrollment was available for 234 of the meningeal TB cases. Cord blood controls (n=759) were recruited at Hung Vuong Obstetric Hospital in Ho Chi Minh City. All control participants were of Vietnamese Kinh ethnicity and had umbilical cord blood samples drawn at birth. All protocols were performed in accordance with human subjects review committees at each site, the Oxford Tropical Research Ethics Committee, the University of Washington (Seattle, WA), and the Western Institutional Review Board (Olympia, WA). Informed consent was obtained in writing by patients if possible, or their relatives or parents, as appropriate.

Leprosy cases (N=899) were recruited from individuals referred for treatment of leprosy at Anandaban hospital in Kathmandu, Nepal. Diagnosis and WHO classification of leprosy into paucibacillary (N=335) multibacillary with no ENL (n=443) and multibacillary with ENL (N=121) classes were made by clinical symptoms, slit skin

smears, and histological criteria. Each individual had a minimum of 9 months of clinical follow-up. Protocols were approved by the Nepal Health Research Council, the University of Washington (Seattle, WA), the University of Medicine and Dentistry of New Jersey (Newark, NJ), and the Western Institutional Review Board (Olympia, WA). Written informed consent was obtained from all patients or from their relatives if the patient could not provide consent.

Genomic DNA was prepared via the QIAamp DNA blood kit (Qiagen) from peripheral blood samples. Seven SNPs were selected for genotyping within the *LTA4H* gene, previously described as part of the HapK haplotype (Helgadottir et al., 2006). Genotyping of SNPs was performed using a MassARRAY technique (Sequenom), as described (Hawn et al., 2006). One SNP failed in >20% of samples and upon repeat on alternate platforms yielded poor quality genotypes. This SNP was dropped from our analysis, leaving six SNPs in the haplotype.

Univariate analysis was performed for allele, genotype, and two-SNP haplotype frequencies with a χ^2 test (or Fisher's exact test when sample number for any group was less than 5). Two-SNP haplotypes for each individual were deduced using the genotyping data at rs1978331 and rs2660898. Individuals heterozygous at both SNPs were compared to individuals not heterozygous at both sites. Logistic regression was used to adjust for gender in the TB cohort, and for gender and age in the leprosy cohort (Stata Intercooled version 11.0, StataCorp, College Station, TX). In the TB cohort, a Bonferroni correction was used to correct for multiple comparisons. Mortality from meningeal TB was calculated by Kaplan Meier analysis (Kaplan and Meier, 1958) and differences in mortality evaluated by log-rank tests (Peto and Peto, 1972).

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