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Supplemental Figure Legends

Figure S1. (Accompanies Figure 1)

(A) MS^3 structural analysis of LXA_4 metabolite. Further fragmentation of m/z 235 (daughter ion) from m/z 351 (parent ion) yielded MS^3 (granddaughter) fragments of m/z 137 and others, consistent with the assigned 15-oxo moiety present in the molecule including reduced double bond and chromatographic behavior . (B) Selected ion chromatograms of monoHETEs (5-, 12- and 15-HETE) from adult zebrafish and MS/MS structural assignments. (C-E) Quantitative profiling of arachidonic acid-derived lipid mediators from adult zebrafish. (C) LTB_4, (D) LXA_4 and (E) monoHETEs. None of the differences for these molecules between wt and mutant were statistically significant. (F,G) Representative lipidomic profiles of zebrafish larvae. (F) Extracted ion chromatograms of 13,14-dihydro-15-oxo-LXA_4 and LTB_4. (G) MS/MS spectra of monoHETEs, e.g. 5-, 12- and 15-HETE.

Figure S2. (Accompanies Figure 2)

lta4h RNA rescues the hypersusceptibility of the *lta4h* morphant in a dose-dependent fashion. FPC at 3 dpi in WT or LTA4H morphant animals injected at one-cell stage with 2-4 nL of *lta4h* sense RNA or *lta4h* antisense RNA as a control at indicated concentrations in ng/uL. Hypersusceptibility observed in LTA4H-low animals was completely rescued by overexpressing wild type lta4h sense RNA in a dose-dependent fashion but not by *lta4h* antisense RNA, confirming the specificity of the morpholino

against *lta4h*. Infection was by intravenous injection with 150-200 Mm. ***, $p<0.001$, all other comparisons not significant (one-way ANOVA followed by Tukey's post-test).

Figure S3. (Accompanies Figure 3)

Recombinant zebrafish TNF rescues the hypersusceptibility of TNF morphants. FPC in WT or TNF morphant animals 4 dpi with ~ 40 Mm with or without injection of 0.5 ng purified recombinant zebrafish TNF 12 hours after infection. Representative of 2 independent experiments. *$P<0.05$; **$P<0.01$ (one-way ANOVA with Tukey's post test).

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ASA inhibits LTB_4-mediated neutrophil migration to ear. Number of neutrophils in ear of 48 hpi larvae with and without soaking in 1 μM ASA (same concentration as used in Figure 4D and 4E) and six hours post-injection of LTB_4 into ear cavity. Details of assay in (Tobin et al., 2010).

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Therapies directed to the LTA4H-low or –high genotypes are not beneficial to WT animals and the benefit of genotype-dependent therapies are abrogated by direct modulation of TNF. (A-D) FPC of WT animals 3dpi with 150-200 Mm treated with the same concentration of each compound used in Figure 4 or vehicle. Representative of 2 independent experiments for each compound. The 15-LOX inhibitor resulted in a slight increase in bacterial burden in the WT, while the other treatments had no effect on bacterial burden. (E-H) Genotype-dependent rescue is abrogated by manipulation of TNF

levels. (E) FPC 3 dpi of control, LTA4H or LTA4H and TNF morphant animals infected with 90-100 Mm and treated with 100 nM 15-LOX inhibitor. (F-H) FPC of 3 dpi of control or LTA4H-high animals injected with 0.5 ng rTNF vehicle followed by no further treatment or treatment with (F) 1 μM ASA, (G) 1 μM LTB_4R antagonist or (H) 0.75 μM dexamethasone. In (E),(F),(G) and (H), the treatment had the expected beneficial effect of lowered bacterial numbers, and this beneficial effect was reversed by the TNF MO in (E) and the rTNF in (F), (G) and (H).

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(A) rs17525495 genotype frequencies and expression levels of *LTA4H* in HapMap LCLs. rs17525495 T is more common among Asians (allele frequency 0.29 in CHB+JPT) than among west Africans (0.12 in YRI) and very rare in Caucasians (0.04 in CEU) (Stranger et al., 2007). (B). LTA4H and β-tubulin protein expression levels from LCLs of unrelated Han Chinese individuals homozygous for the C or T allele or CT heterozygotes detected by Western blot. (C) Luciferase expression (mean ± SEM of 3 independent experiments) transcribed from a one kb promoter fragment immediately upstream of the *LTA4H* translation start site containing the rs17525495 C and T alleles. P=0.03 for uninfected cells.

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Table S1. 182 Vietnamese HIV uninfected adults with microbiologically confirmed TB meningitis: description of the studies contributing to the current cohort analyzed.

Study reference	Dates of recruitment	Number of patients*	Study Design	Anti-TB therapy	Inclusions	Adjunctive Dexamethasone (DEX)	Follow-up
(Thwaites et al., 2003)	2000-2001	20	Prospective observational	4 drugs 3 months; 2 drugs 6 months	Adults (>14 years) with clinically suspected TB meningitis	DEX not given to any patient	9 months
(Thwaites et al., 2004)	2001-2003	109	Prospective randomized placebo controlled trial	4 drugs 3 months; 2 drugs 6	Adults (>14 years) with clinically	Randomly allocated to DEX or placebo (1:1 ratio)	9 months

				months	suspected TB meningitis		
(Thwaites et al., 2011)	2003-2006	53	Prospective observational	5 drugs 3 months; 2 drugs 6 months	Adults (>14 years) with clinically suspected TB meningitis	DEX given to all patients	9 months

*Number of patients in each study from whom DNA was collected at the start of the study

Table S2. Comparison of pre-treatment clinical variables by genotype and dexamethasone treatment (N=182)

Variable	CC genotype (n=84) *Median (range), no. (%)*	CT (n=73) *Median (range), no. (%)*	TT (n=25) *Median (range), no. (%)*	P-value[a]
Age (years)	36 (15-83)	31 (16-74)	33 (19-68)	0.319
Male sex	45 (53.6)	35 (47.9)	13 (52.0)	0.777
Duration of symptoms (days)	15 (3-180)	17 (5-60)	14 (6-90)	0.688
Glasgow Coma Score (/15) [b]	13 (3-15)	14 (6-15)	13 (3-15)	0.435
MRC grade [c]: 1 2 3	 19 (22.6) 40 (47.6) 25 (29.8)	 20 (27.8) 34 (47.2) 18 (25.0)	 5 (20.0) 12 (48.0) 8 (32.0)	 0.892
Temperature (^{O}C)	38.0 (37.0-40.0)	38.5 (37.0-40.2)	38.5 (37.0-40.6)	0.350
CSF Opening pressure (cm H_20)	20 (1-44)	25 (7-41)	27 (14-44)	0.287
CSF Total leukocytes ($/mm^3$)	155 (3-1030)	248 (5-1880)	345 (21-1770)	0.022
CSF Total	160 (31-1800)	161 (36-2500)	126 (23-360)	0.264

protein (mg/dl)				
CSF:plasma glucose conc.	0.26 (0.02-0.66)	0.26 (0.05-0.67)	0.27 (0.07-0.53)	0.761
CSF microbiology: Smear +, Culture + Smear -, Culture + Smear +, Culture -	 27 (32.1) 53 (63.1) 4 (4.8)	 23 (31.9) 43 (59.7) 6 (8.3)	 10 (40.0) 12 (48.0) 3 (12.0)	0.418
	Dexamethasone treated (n=55)	Dexamethasone treated (n=46)	Dexamethasone treated (13)	
MRC grade: 1 2 3	 12 (21.8) 27 (49.1) 16 (29.1)	 14 (31.1) 24 (53.3) 7 (15.6)	 5 (38.5) 6 (46.2) 2 (15.4)	0.420

[a] Categorical variables compared by Chi Squared test; continuous variables by Mann-Whitney U test.

[b] Glasgow coma score (GCS) is used to define the severity of coma by assessing the best eye (1-4 points), verbal (1-5 points), and motor (1-6 points) responses to stimulation. The maximum number of points (15) indicates full consciousness; the minimum points (3) indicates deep coma (Teasdale and Jennett, 1974).

[c] Disease severity grade From the British Medical Research Council (MRC). Grade I = Alert and orientated and no focal neurological signs. Grade II = disorientated (GCS 11-14) and/or focal neurological signs. Grade III = comatose (GCS <11) with or without focal neurological signs (British Medical Council (1948).

Extended Experimental Procedures

Bacterial Strains

Wildtype strain M (ATCC #BAA-535) was transformed with plasmids containing transcriptional fusions of the gene encoding Wasabi to a constitutively-expressed Mm promoter as described (Adams et al., 2011). The *erp* mutant expressing *msp-12::gfp* (Cosma et al., 2006b) was used for quantitation of intracellular bacterial burdens in Fig.

2D. Bacteria were grown in 7H9 media supplemented with oleic acid-albumin-dextrose-complex and 0.05% Tween-80 unless otherwise stated.

Embryo Infection and Husbandry

All zebrafish experiments were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals using protocols approved by the Institutional Animal Care and Use Committee of the University of Washington. Zebrafish embryos of the AB line were injected with Mm or PBS (mock-injected) using phenol red as a visual marker at 30 hours post fertilization or 48 hours post fertilization (hpf) via caudal vein as described (Cosma et al., 2006a). Hindbrain ventricle injections were performed as described at 24 hpf (Davis et al., 2002). Innocula at injection were determined injecting the same volume onto selective bacteriologic plates and enumerating bacteria when colonies formed in 5-7 days. Bacterial burdens of larvae were determined by fluorescence pixel counts (FPC) (Adams et al., 2011).

Eicosanoid Extraction and LC-MS-MS lipid mediator lipidomics

Adult zebrafish from an incross of *lta4h*^{zm5961}/+ heterozygotes were identified by PCR as in (Tobin et al., 2010). Three wildtype homozygotes and three *zm5961/zm5961* siblings were euthanized and snap frozen then precipitated with ice-cold methanol. For larvae sample preparation, 900 larvae of each condition were euthanized and homogenized in ice-cold methanol with addition of 500 picograms of internal standard mixture containing d_8-5S-HETE, d_4-LTB_4 and d_4-PGE_2 each label was from Cayman Chemical and calibration curves were obtained for each LC-chromatographic region to calculate

relative amounts and recoveries for each extraction . Samples were extracted using Waters C18 solid-phase extraction (SPE) column as described in (Yang et al., 2011). Extracted samples were injected to either ABI Qtrap (AB Sciex) 3200 equipped with Shimadzu AC-20 binary pump and autosampler (for quantitation) or ABI Qtrap 5500 with Agilent HP1100 binary pump (for MS^3 analysis and high-sensitivity identification) system, using Agilent Eclipse C18 (50mm x 4.6mm x 2.1um) column with gradient elution from methanol:water:acetic acid=55:45:0.01 to 100:0:0.01 at 0.5mL/min flow rate. A list of parent-signature ion pairs was developed and used for multiple reaction monitoring (MRM) and information-dependent acquisition (IDA) of tandem mass spectra. For MS^3 analysis, samples were pre-fractionated and directly infused with built-in syringe pump. Structures of lipid mediators were assigned in accordance with published established criteria (Yang et al. 2011).

LXA_4 metabolite, namely 13,14-dihydro-15-oxo LXA4, from zebrafish was isolated by RP-HPLC (Agilent HP1100 binary pump with Agilent Eclipse C18 column) with similar gradient program used for LC-MS-MS analysis (see above) . The material in a single peak gave characteristic UV chromophore at 272nm was isolated, concentrated with a stream of nitrogen gas and quantified by calculating absorbance with HP8453 UV-VIS spectrometer. For this product an extinction coefficient of a typical conjugated triene e=50,000 was used to determine the concentration of the isolated LXA4 metabolite from zebrafish.

Quantitative RT-PCR

Total RNA obtained by Trizol extraction from infected or mock-injected embryos were used as templates for generating cDNA (Superscript II reverse transcriptase; random

hexamer primers; Invitrogen) for quantitative real-time RT-PCR analysis. qRT-PCR assays were performed such that each 20μL reaction contained either 250 nM of gene-specific primers or b-actin specific control primers. SYBR green PCR Master Mix (Applied Biosystems) was used at 1X. All qRT-PCR assays were performed in triplicate with an ABI Prism 7300 Real Time PCR System (Applied Biosystems). Data were normalized to b-actin (ΔΔCT analysis).

Morpholino and RNA injections

Morpholinos were obtained from Genetools (Eugene, OR). Control, *lta4h* and *tnf* morpholinos were as previously described (Lopez-Munoz et al., 2011; Tobin et al., 2010), with an injection volume of 2-4 nL of 200 μM *lta4h* morpholino and 2-4 nL of 200 μM morpholino for *tnf*. All morpholino infections were performed on paired needles; an equal number of morpholino and control animals were injected on each needle in an alternating sequence to reduce variations in dosage between groups.

lta4h RNA was synthesized using the mMessage mMachine kit (Ambion) and the polyA Tailing kit (Ambion). 2-4 nL of RNA was injected at one-four cell stage at a concentration of 200 ng/μL to create LTA4H-high animals.

Recombinant TNF microinjection

Vehicle, 0.5 ng or 1.0 ng of recombinant zebrafish TNF (Roca et al., 2008) was microinjected into the caudal vein of each animal at 16 hpi.

Pharmacological interventions in zebrafish

After infection, small molecules were applied via soaking. Solutions were changed daily. All conditions and controls were standardized to a final concentration of 0.5% DMSO. PD 146176 (BIOMOL) was applied at a final concentration of 100 nM 16 hpi. U75302 (BIOMOL) was applied at a concentration of 1 μM directly after infection. Dexamethasone (Sigma) was applied at a concentration of 0.75 μM directly after infection. Acetylsalicylic acid (Sigma) was applied at a concentration of 1 μM.

A single dose of approximately 5 nL of Lipoxin A_4 or its metabolite 13,14-dihydro-15-oxo-LXA_4 or vehicle control was microinjected into 3 dpi animals (30 for each condition) at a concentration of 0.2 ng/μL in 10% ethanol. Seven hours later, animals were harvested to measure TNF mRNA levels.

Microscopy

Microscopy was performed on a Nikon E600 equipped with DIC optics, a Nikon D-FL-E fluorescence unit with 100W Mercury lamp and MFC-1000 z-step controller (Applied Scientific Instrumentation) or, for whole animal images, a motorized Nikon inverted Ti-E microscope. Objectives used included 2x Apo Objective 0.1 NA, 10x Plan Fluor 0.3 NA, 40x Plan Fluor 0.75 NA and 60x Oil Plan Apo, 1.4 NA. Widefield fluorescence were captured on a CoolSnap HQ or CoolSnap CF CCD camera (Photometrics) using MetaMorph 7.1 (Molecular Devices) and Elements AR software. Confocal images were obtained using a Nikon A1RSi confocal and Nikon Elements AR software

Neutral Red Staining

Neutral red staining was performed as described (Tobin et al., 2010).

Quantitation of LTA4H *protein levels*

Indicated lymphoblastoid cell lines (LCLs) from Han Chinese in the HapMap project were split to $2x10^5$/ml and grown for 3 days. Cells were counted, lysed in RIPA buffer, and lysate from $2x10^6$ cells was loaded in each lane. Blots were probed first with anti-LTA4H (Santa Cruz Biotechnology, 1:50 dilution) and then with anti-btubulin (Developmental Studies Hybridoma Bank 1:500) and exposed to film for varying amounts of time. Films were scanned and ImageJ used to quantify intensity of bands. Mean-centered values for LTA4H and b-Tubulin were obtained for two independent experiments.

LTA4H promoter luciferase assays

A 1068 nucleotide fragment immediately upstream of the *LTA4H* translational start site was cloned into the promoterless luciferase vector pGL4.10, to drive the reporter gene *luc2*. This construct was co-transfected with pGL4.74 (hRLuc/TK), which drives *Renilla reniformis* luciferase from a thymidine kinase promoter and is used as an internal normalization for transfection efficiency. Transfections were performed on RAW 264.7 and THP-1 macrophages using the Amaxa nucleofection system and according to the recommended protocols of the manufacturer. To study the effects of infection, macrophages were infected with Mm at a multiplicity of infection of 0.1 six to eight hours after transfection. The ratio of *luc2* luciferase activity to *Renilla* luciferase determined at 18 hours post-transfection.

LTA4H allelic imbalance assay

For the assay shown in Figure 6C, using available sequence data from the 1000 genomes project, we identified an LCL (NA19190) that was heterozygous for both the rs17525495 SNP and the rare SNP rs79510571, which lies within the coding region and thus could be used to distinguish which chromosome a given RNA was derived from. Because the promoter SNP and coding sequence SNP lie only 90 bp apart, we could also determine phase for this LCL by sequencing individual clones from genomic DNA. We then isolated and sequenced individual cDNA clones derived from RNA extracted from this LCL to determine the relative abundance of RNA derived from each chromosome.

Clinical methods

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.

Consecutively treated, HIV uninfected adults (>14 years old) with microbiologically confirmed (*M. tuberculosis* seen or cultured from the cerebrospinal fluid) TB meningitis were recruited from two hospitals in Ho Chi Minh City, Vietnam: the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease. Participants in the current study (n=182) were recruited from one of three consecutive, prospective clinical studies (Table S1). In each, methods for recording clinical data and assessing outcome were identical and details have been described previously (Table S2) (Thwaites et al., 2011; Thwaites et al., 2004; Thwaites et al.,

2003). All patients received 9 months of anti-tuberculosis chemotherapy; however, the use of adjunctive dexamethasone differed for each study. None of the patients recruited from the first study (2000-2001) received dexamethasone (n=20), as it was not the standard of care. Patients in the second study (2001-2003) were randomized to either adjunctive dexamethasone or identical placebo in a 1:1 ratio (n=109) (Thwaites et al., 2004). The results of this study demonstrated adjunctive dexamethasone improved survival from TB meningitis; therefore all patients received dexamethasone in the studies that followed (2003-2006) (n=53). Selection bias on the basis of dexamethasone treatment could not operate in the studies performed before and after the controlled trial because none of the patients received dexamethasone in the first study and all received it in the third.

Genomic DNA was prepared via the QIAamp DNA blood kit (Qiagen) from peripheral blood samples. Genotype at rs17525495 was determined after PCR amplification of a 280 bp fragment containing the SNP of interest followed by Qiagen spin column purification and sequencing.

Statistical Analysis

Statistical analysis was performed with Prism (Graphpad Software) and STATA (Statacorp LP, Texas, USA) and is detailed in each figure legend.

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Male sex	45 (53.6)	35 (47.9)	13 (52.0)	0.777
Duration of symptoms (days)	15 (3-180)	17 (5-60)	14 (6-90)	0.688
Glasgow Coma Score (/15) [b]	13 (3-15)	14 (6-15)	13 (3-15)	0.435
MRC grade [c]: 1 2 3	 19 (22.6) 40 (47.6) 25 (29.8)	 20 (27.8) 34 (47.2) 18 (25.0)	 5 (20.0) 12 (48.0) 8 (32.0)	 0.892
Temperature (^{O}C)	38.0 (37.0-40.0)	38.5 (37.0-40.2)	38.5 (37.0-40.6)	0.350
CSF Opening pressure (cm H_20)	20 (1-44)	25 (7-41)	27 (14-44)	0.287
CSF Total leukocytes	155 (3-1030)	248 (5-1880)	345 (21-1770)	0.022

(/mm^3)				
CSF Total protein (mg/dl)	160 (31-1800)	161 (36-2500)	126 (23-360)	0.264
CSF:plasma glucose conc.	0.26 (0.02-0.66)	0.26 (0.05-0.67)	0.27 (0.07-0.53)	0.761
CSF microbiology: Smear +, Culture + Smear -, Culture + Smear +, Culture -	 27 (32.1) 53 (63.1) 4 (4.8)	 23 (31.9) 43 (59.7) 6 (8.3)	 10 (40.0) 12 (48.0) 3 (12.0)	0.418
	Dexamethasone treated (n=55)	Dexamethasone treated (n=46)	Dexamethasone treated (13)	
MRC grade: 1 2 3	 12 (21.8) 27 (49.1) 16 (29.1)	 14 (31.1) 24 (53.3) 7 (15.6)	 5 (38.5) 6 (46.2) 2 (15.4)	0.420

[a] Categorical variables compared by Chi Squared test; continuous variables by Mann-Whitney U test.

[b] Glasgow coma score (GCS) is used to define the severity of coma by assessing the best eye (1-4 points), verbal (1-5 points), and motor (1-6 points) responses to stimulation. The maximum number of points (15) indicates full consciousness; the minimum points (3) indicates deep coma (Teasdale and Jennett, 1974).

[c] Disease severity grade From the British Medical Research Council (MRC). Grade I = Alert and orientated and no focal neurological signs. Grade II = disorientated (GCS 11-14) and/or focal neurological signs. Grade III = comatose (GCS <11) with or without focal neurological signs (British Medical Council (1948).

Extended Experimental Procedures

Bacterial Strains

Wildtype strain M (ATCC #BAA-535) was transformed with plasmids containing transcriptional fusions of the gene encoding Wasabi to a constitutively-expressed Mm promoter as described (Adams et al., 2011). The *erp* mutant expressing *msp-12::gfp* (Cosma et al., 2006b) was used for quantitation of intracellular bacterial burdens in Fig.

2D. Bacteria were grown in 7H9 media supplemented with oleic acid-albumin-dextrose-complex and 0.05% Tween-80 unless otherwise stated.

Embryo Infection and Husbandry

All zebrafish experiments were conducted in conformity with the Public Health Service Policy on Humane Care and Use of Laboratory Animals using protocols approved by the Institutional Animal Care and Use Committee of the University of Washington. Zebrafish embryos of the AB line were injected with Mm or PBS (mock-injected) using phenol red as a visual marker at 30 hours post fertilization or 48 hours post fertilization (hpf) via caudal vein as described (Cosma et al., 2006a). Hindbrain ventricle injections were performed as described at 24 hpf (Davis et al., 2002). Innocula at injection were determined injecting the same volume onto selective bacteriologic plates and enumerating bacteria when colonies formed in 5-7 days. Bacterial burdens of larvae were determined by fluorescence pixel counts (FPC) (Adams et al., 2011).

Eicosanoid Extraction and LC-MS-MS lipid mediator lipidomics

Adult zebrafish from an incross of *lta4h*^{zm5961}/+ heterozygotes were identified by PCR as in (Tobin et al., 2010). Three wildtype homozygotes and three *zm5961/zm5961* siblings were euthanized and snap frozen then precipitated with ice-cold methanol. For larvae sample preparation, 900 larvae of each condition were euthanized and homogenized in ice-cold methanol with addition of 500 picograms of internal standard mixture containing d_8-5S-HETE, d_4-LTB_4 and d_4-PGE_2 each label was from Cayman Chemical and calibration curves were obtained for each LC-chromatographic region to calculate

relative amounts and recoveries for each extraction . Samples were extracted using Waters C18 solid-phase extraction (SPE) column as described in (Yang et al., 2011). Extracted samples were injected to either ABI Qtrap (AB Sciex) 3200 equipped with Shimadzu AC-20 binary pump and autosampler (for quantitation) or ABI Qtrap 5500 with Agilent HP1100 binary pump (for MS^3 analysis and high-sensitivity identification) system, using Agilent Eclipse C18 (50mm x 4.6mm x 2.1um) column with gradient elution from methanol:water:acetic acid=55:45:0.01 to 100:0:0.01 at 0.5mL/min flow rate. A list of parent-signature ion pairs was developed and used for multiple reaction monitoring (MRM) and information-dependent acquisition (IDA) of tandem mass spectra. For MS^3 analysis, samples were pre-fractionated and directly infused with built-in syringe pump. Structures of lipid mediators were assigned in accordance with published established criteria (Yang et al. 2011).

LXA_4 metabolite, namely 13,14-dihydro-15-oxo LXA4, from zebrafish was isolated by RP-HPLC (Agilent HP1100 binary pump with Agilent Eclipse C18 column) with similar gradient program used for LC-MS-MS analysis (see above) . The material in a single peak gave characteristic UV chromophore at 272nm was isolated, concentrated with a stream of nitrogen gas and quantified by calculating absorbance with HP8453 UV-VIS spectrometer. For this product an extinction coefficient of a typical conjugated triene e=50,000 was used to determine the concentration of the isolated LXA4 metabolite from zebrafish.

Quantitative RT-PCR

Total RNA obtained by Trizol extraction from infected or mock-injected embryos were used as templates for generating cDNA (Superscript II reverse transcriptase; random

hexamer primers; Invitrogen) for quantitative real-time RT-PCR analysis. qRT-PCR assays were performed such that each 20μL reaction contained either 250 nM of gene-specific primers or b-actin specific control primers. SYBR green PCR Master Mix (Applied Biosystems) was used at 1X. All qRT-PCR assays were performed in triplicate with an ABI Prism 7300 Real Time PCR System (Applied Biosystems). Data were normalized to b-actin (ΔΔCT analysis).

Morpholino and RNA injections

Morpholinos were obtained from Genetools (Eugene, OR). Control, *lta4h* and *tnf* morpholinos were as previously described (Lopez-Munoz et al., 2011; Tobin et al., 2010), with an injection volume of 2-4 nL of 200 μM *lta4h* morpholino and 2-4 nL of 200 μM morpholino for *tnf*. All morpholino infections were performed on paired needles; an equal number of morpholino and control animals were injected on each needle in an alternating sequence to reduce variations in dosage between groups.

lta4h RNA was synthesized using the mMessage mMachine kit (Ambion) and the polyA Tailing kit (Ambion). 2-4 nL of RNA was injected at one-four cell stage at a concentration of 200 ng/μL to create LTA4H-high animals.

Recombinant TNF microinjection

Vehicle, 0.5 ng or 1.0 ng of recombinant zebrafish TNF (Roca et al., 2008) was microinjected into the caudal vein of each animal at 16 hpi.

Pharmacological interventions in zebrafish

After infection, small molecules were applied via soaking. Solutions were changed daily. All conditions and controls were standardized to a final concentration of 0.5% DMSO. PD 146176 (BIOMOL) was applied at a final concentration of 100 nM 16 hpi. U75302 (BIOMOL) was applied at a concentration of 1 μM directly after infection. Dexamethasone (Sigma) was applied at a concentration of 0.75 μM directly after infection. Acetylsalicylic acid (Sigma) was applied at a concentration of 1 μM.

A single dose of approximately 5 nL of Lipoxin A_4 or its metabolite 13,14-dihydro-15-oxo-LXA_4 or vehicle control was microinjected into 3 dpi animals (30 for each condition) at a concentration of 0.2 ng/μL in 10% ethanol. Seven hours later, animals were harvested to measure TNF mRNA levels.

Microscopy

Microscopy was performed on a Nikon E600 equipped with DIC optics, a Nikon D-FL-E fluorescence unit with 100W Mercury lamp and MFC-1000 z-step controller (Applied Scientific Instrumentation) or, for whole animal images, a motorized Nikon inverted Ti-E microscope. Objectives used included 2x Apo Objective 0.1 NA, 10x Plan Fluor 0.3 NA, 40x Plan Fluor 0.75 NA and 60x Oil Plan Apo, 1.4 NA. Widefield fluorescence were captured on a CoolSnap HQ or CoolSnap CF CCD camera (Photometrics) using MetaMorph 7.1 (Molecular Devices) and Elements AR software. Confocal images were obtained using a Nikon A1RSi confocal and Nikon Elements AR software

Neutral Red Staining

Neutral red staining was performed as described (Tobin et al., 2010).

Quantitation of LTA4H *protein levels*

Indicated lymphoblastoid cell lines (LCLs) from Han Chinese in the HapMap project were split to $2x10^5$/ml and grown for 3 days. Cells were counted, lysed in RIPA buffer, and lysate from $2x10^6$ cells was loaded in each lane. Blots were probed first with anti-LTA4H (Santa Cruz Biotechnology, 1:50 dilution) and then with anti-btubulin (Developmental Studies Hybridoma Bank 1:500) and exposed to film for varying amounts of time. Films were scanned and ImageJ used to quantify intensity of bands. Mean-centered values for LTA4H and b-Tubulin were obtained for two independent experiments.

LTA4H promoter luciferase assays

A 1068 nucleotide fragment immediately upstream of the *LTA4H* translational start site was cloned into the promoterless luciferase vector pGL4.10, to drive the reporter gene *luc2*. This construct was co-transfected with pGL4.74 (hRLuc/TK), which drives *Renilla reniformis* luciferase from a thymidine kinase promoter and is used as an internal normalization for transfection efficiency. Transfections were performed on RAW 264.7 and THP-1 macrophages using the Amaxa nucleofection system and according to the recommended protocols of the manufacturer. To study the effects of infection, macrophages were infected with Mm at a multiplicity of infection of 0.1 six to eight hours after transfection. The ratio of *luc2* luciferase activity to *Renilla* luciferase determined at 18 hours post-transfection.

LTA4H allelic imbalance assay

For the assay shown in Figure 6C, using available sequence data from the 1000 genomes project, we identified an LCL (NA19190) that was heterozygous for both the rs17525495 SNP and the rare SNP rs79510571, which lies within the coding region and thus could be used to distinguish which chromosome a given RNA was derived from. Because the promoter SNP and coding sequence SNP lie only 90 bp apart, we could also determine phase for this LCL by sequencing individual clones from genomic DNA. We then isolated and sequenced individual cDNA clones derived from RNA extracted from this LCL to determine the relative abundance of RNA derived from each chromosome.

Clinical methods

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.

Consecutively treated, HIV uninfected adults (>14 years old) with microbiologically confirmed (*M. tuberculosis* seen or cultured from the cerebrospinal fluid) TB meningitis were recruited from two hospitals in Ho Chi Minh City, Vietnam: the Hospital for Tropical Diseases and Pham Ngoc Thach Hospital for Tuberculosis and Lung Disease. Participants in the current study (n=182) were recruited from one of three consecutive, prospective clinical studies (Table S1). In each, methods for recording clinical data and assessing outcome were identical and details have been described previously (Table S2) (Thwaites et al., 2011; Thwaites et al., 2004; Thwaites et al.,

2003). All patients received 9 months of anti-tuberculosis chemotherapy; however, the use of adjunctive dexamethasone differed for each study. None of the patients recruited from the first study (2000-2001) received dexamethasone (n=20), as it was not the standard of care. Patients in the second study (2001-2003) were randomized to either adjunctive dexamethasone or identical placebo in a 1:1 ratio (n=109) (Thwaites et al., 2004). The results of this study demonstrated adjunctive dexamethasone improved survival from TB meningitis; therefore all patients received dexamethasone in the studies that followed (2003-2006) (n=53). Selection bias on the basis of dexamethasone treatment could not operate in the studies performed before and after the controlled trial because none of the patients received dexamethasone in the first study and all received it in the third.

Genomic DNA was prepared via the QIAamp DNA blood kit (Qiagen) from peripheral blood samples. Genotype at rs17525495 was determined after PCR amplification of a 280 bp fragment containing the SNP of interest followed by Qiagen spin column purification and sequencing.

Statistical Analysis

Statistical analysis was performed with Prism (Graphpad Software) and STATA (Statacorp LP, Texas, USA) and is detailed in each figure legend.

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