Editor's Summary

A Screening Room for Cerebral Malaria Drugs

Smart-phone retailers claim that their device brings the cinema to you. Yet, there's something to be said for ambiance: A blockbuster film delivers more effective entertainment when screened in high-def surround sound versus on a 6-inch screen in a jammed subway car. Screening new drugs is no different. Although in vitro drug-screening models are convenient, it's attention to pathophysiological details that creates an informative and predictive drug-screening platform. Achtman et al. now repurpose a mouse model of experimental cerebral malaria (ECM) to screen for adjuvant therapy against severe malaria.

Although first-line antimalarial drugs may control parasitemia, they do little to quell the inflammation that leads to seizures, coma, and long-term sequelae in patients with severe malaria. The authors hypothesized that innate defense regulator (IDR) peptides—host-derived signaling molecules that modulate the innate immune system—would serve as anti-inflammatory adjuvant therapy for ECM in combination with first-line drugs. The authors adapted the murine Plasmodium berghei ANKA ECM model to serve as a preclinical drug-screening platform by combining antimalarial drug intervention in established malarial infections with iterative mouse-human bioinformatic analysis. When co-administered with first-line antimalarial drugs, the IDR peptide down-regulated inflammatory networks and improved mouse survival. If these data translate to humans, IDR peptides could provide an anti-inflammatory adjuvant therapy for severe malaria and perhaps other infectious diseases.
MALARIA

Effective Adjunctive Therapy by an Innate Defense Regulatory Peptide in a Preclinical Model of Severe Malaria

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Case fatality rates for severe malaria remain high even in the best clinical settings because antimalarial drugs act against the parasite without alleviating life-threatening inflammation. We assessed the potential for host-directed therapy of severe malaria of a new class of anti-inflammatory drugs, the innate defense regulator (IDR) peptides, based on host defense peptides. The Plasmodium berghei ANKA model of experimental cerebral malaria was adapted to use as a preclinical screen by combining late-stage intervention in established infections with advanced bioinformatic analysis of early transcriptional changes in co-regulated gene sets. Coadministration of IDR-1018 with standard first-line antimalarials increased survival of infected mice while down-regulating key inflammatory networks associated with fatality. Thus, IDR peptides provided host-directed adjunctive therapy for severe disease in combination with antimalarial treatment.

INTRODUCTION

Clinically severe or life-threatening malaria presents a global health challenge. Clinical symptoms include seizures and coma, with long-term neurological and physical sequelae in survivors. Diverse organ-specific or systemic disease syndromes in Plasmodium falciparum infection are thought to represent the end-stage processes of parasite sequestration in local sites and inflammatory cascades initiated by pathogen toxins. The only effective treatment for clinically severe malaria is parenteral administration of antimalarial drugs (1), which control parasitemia but do little to reverse pathophysiological processes already under way in the brain or other organs. Various adjunctive therapies assessed in preclinical models or small clinical trials (1) either were ineffective or provided contradictory outcomes. The rational development of supportive or adjunctive therapies is hindered by both lack of clarity as to underlying pathologic mechanisms in severe malaria and a failure to develop appropriate preclinical screens. The relevance of the murine Plasmodium berghei ANKA experimental cerebral malaria (ECM) model to human disease has been extensively debated (2–8). Differences in opinion revolve around two issues, namely, the relative contributions to these syndromes of parasite sequestration and host inflammation, and the reliance on early-stage rather than late-stage interventions in exploiting the model (2–8). In addition to extensive data linking proinflammatory responses to malaria disease states (9–13), unbiased and hypothesis-driven gene association studies have identified immune regulatory and effector loci as a major ontological class of mutations associated with susceptibility and resistance to human severe malaria (14). These findings justify research into anti-inflammatory drugs for the supportive therapy of severe malaria.

Host defense peptides (such as defensins or cathelicidins) and their synthetic innate defense regulator (IDR) analogs modulate innate immunity. Their modes of action include chemokine secretion, cell differentiation, and the suppression of potentially harmful inflammatory responses (15–17). In addition, they enhance adaptive immune responses (18). These multiple modes of action make host defense peptides attractive tools in combating a multifactorial syndrome such as cerebral malaria (CM). Accordingly, we sought to use the ECM system to assess late-stage intervention in combination with first-line antimalarial treatment as a preclinical screen for the therapeutic efficacy of IDR-1018 as a host-directed therapy and adjunctive treatment for severe malaria. Adjunctively administered IDR-1018 was significantly more protective against late-stage infection than antimalarial treatment alone, and this was linked to reduced inflammation.

RESULTS

Prophylactic administration of IDR-1018 peptide reduced the incidence of ECM

Peptide IDR-1018, based loosely on Bac2a (fig. S1A), the linear form of the bovine host defense peptide bactenecin, was selected for its broad anti-inflammatory capabilities. IDR-1018 suppressed lipopolysaccharide (LPS)–induced production of tumor necrosis factor–α (TNF-α) in vitro and protected mice from Staphylococcus aureus infection (fig. S1B). The peptide’s potent ability to modulate inflammatory immune responses and its low toxicity profile indicated its potential as an adjunctive therapy for malaria treatment. IDR-1 [fig. S1A (16)], a less potent immune modulator, was used as a control.

In the P. berghei ANKA/C57BL/6 model of ECM, 90 to 100% of mice die from inflammatory processes in the brain about 6 to 8 days...
after infection, with comparatively low parasite burdens (~10 to 15%). We first tested intraperitoneal and intravenous prophylactic administration of IDR-1018 (Fig. 1A). The intraperitoneal treatment with IDR-1018 peptide was not protective, with 100% of mice in this group dying of ECM at rates comparable to those of saline-injected controls (93%) (Fig. 1B). However, 56% of intravenous IDR-1018–treated mice were protected (Fig. 1B, P = 0.0019). Although mice were treated with IDR-1018 only up to day 9 of infection, there was no late recurrence of ECM symptoms.

Drugs can protect against ECM by reducing either parasitemia or pathology. Alterations in reticulocyte levels may have downstream effects on parasitemia because they are the preferred invasion target for *P. berghei* in late-stage infections. Reticulocyte levels were significantly (P = 0.004) higher in IDR-1018 intravenously treated mice than in saline-treated animals on day 3 after infection (Fig. 1C), but this effect did not persist. Moreover, IDR-1018 treatment did not affect parasitemia (Fig. 1D), suggesting that the peptide acts in a host-directed manner.

**IDR-1018 adjunctive treatment increased protection from ECM**

IDR-1018 was tested in an adjunctive treatment regimen (Fig. 2). To approximate the clinical setting, where treatment occurs after admission, we commenced intravenous IDR-1018 treatment in combination with antimalarial drugs on day 4 of infection, when the animals manifested peripheral parasitemias of 3 to 6% (Fig. 2A). All saline-treated mice developed fatal ECM by day 7 after infection (Fig. 2, B and C). Treatment of an established infection with IDR-1018 without antimalarials neither slowed down nor prevented ECM development (Fig. 2B). Pyrimethamine-chloroquine antimalarial treatment of established infections protected only 41% of mice from ECM. Protection from ECM was significantly (P = 0.034) boosted to 68% by adjunctive treatment with IDR-1018 peptide together with antimalarial drugs (Fig. 2C). In contrast, the control peptide IDR-1 (16) failed to protect in this model, even though it exhibited some protection prophylactically (Fig. 2D).

**Malaria infection and treatment with IDR-1018 created distinct transcriptional profiles**

Microarray analyses were performed to provide mechanistic insights into the protective effect of IDR-1018 against ECM. The microarrays compared nine IDR-1018 intravenously treated, infected mice (IDR-1018–treated infected) with three saline intravenously treated, infected mice (saline-treated infected) and three uninfected untreated mice (controls). In ECM, the spleen is the origin of immunopathological processes and the brain is the site of terminal events. To detect both early-onset causal processes and pathology, we administered IDR-1018 prophylactically and harvested spleens and brains on day 3 of infection (Fig. 3A). In our past ECM experiments, a group size of three has produced consistent microarray profiles (19), but we expected high transcriptional diversity in the IDR-1018–treated infected group because of incomplete penetrance of the protective phenotype. We compensated by tripling the size of the IDR-1018–treated infected group.

An unsupervised plot was used to display the overall relatedness of the 15 microarray expression profiles for each tissue. For each pair of samples, the leading fold change was computed as the typical log₂ fold change of the 500 most discrepant genes between those samples. Then,
multidimensional scaling was used to construct a two-dimensional plot of the samples in which the distances between points correspond to leading log2 fold change for the 500 genes that best distinguish each pair of samples.

Fig. 3. IDR-1018 treatment had a transcriptional impact during infection. (A) Prophylactic peptide administration and infection protocol. The efficacy cohort was tracked until day 14, whereas all microarray organs were harvested 3 days after infection. (B and C) Multidimensional scaling plots show relationships between the transcriptional profiles of all samples from either spleen (B) or brain (C). Dimensions 1 and 2 represent distances between samples, which can be interpreted as average log2 fold change for the 500 genes that best distinguish each pair of samples.

In contrast, a network of genes associated with erythropoiesis was up-regulated in the IDR-1018–treated compared to the saline-treated infected spleens (Fig. 4B). This included six of the eight genes for key enzymes in heme biosynthesis as well as central regulators of erythropoiesis such as GATA-1 and EPO-R. SOCS3 and HMAG1 were the only examples of these genes differentially expressed (up-regulated) between saline-treated infected and uninfected spleens. Further up-regulated genes fell into the functional categories of cell cycle, DNA replication, and DNA repair. Pathways regulating the G2-M and G1-S transitions of the cell cycle and DNA repair in response to cell stress or injury were statistically overrepresented in network analysis (summarized in Fig. 4C). Other statistically overrepresented pathways included metabolic pathways related to pyrimidine metabolism and glutathione metabolism. ABC (adenosine triphosphate–binding cassette) transporters were also overrepresented.

To address the incomplete penetration of survival in IDR-1018–treated infected mice, we examined variability among transcriptional profiles of the nine treated mice. To focus on genes relevant to ECM, we undertook a linear discriminant analysis to distinguish the uninfected from the saline-treated infected expression profiles. This analysis produced discriminant functions (or metagenes), weighted averages of the individual genes, that best separated the uninfected and saline-treated infected groups. All the expression profiles, including those for IDR-1018–treated mice, were displayed in a scatterplot according to the first two discriminant functions. Applied to splenic expression profiles, this semisupervised analysis showed that the IDR-1018–treated mice were intermediate between and more heterogeneous than either the uninfected or the saline-treated infected groups (Fig. 5A). Genes that received positive weights in the first discriminant function, and hence were associated with ECM, were dominated by IFN-γ and a range of genes associated either with IFN-γ (GBP2, WARS, Ilrnm1, IL-7, Serpina3f, and Nlrps5) or both type I and type II IFNs (Ifi205, Ifi1, Gbp10, Oasl1g, and Stat1) (Table 1). The second discriminant function was almost entirely determined by IFN-γ.

In the brain transcriptome, the only gene differentially expressed between infected IDR-1018–treated and saline-treated mice was Hbb-b1 (up-regulated), which encodes hemoglobin B1. This limited differential expression was consistent with the poor separation of the groups in the brain multidimensional scaling plot (Fig. 5C). To focus more closely on the ECM phenotype, we examined a set of genes that, according to three published studies (19–21), have altered brain expression in P. berghei–infected, ECM-susceptible mice early in infection (see Materials and Methods). Each gene in the set was classed as either positively or negatively associated with ECM (table S1). Collectively, these genes were used to represent the transcriptional signature of the ECM phenotype. The behavior of the signature was examined using a gene set test method (ROAST) that assesses the significance of changes in the ECM signature as a unit (22).

With this statistically powerful method, it became possible to identify collective trends of co-regulated genes regardless of individual P values. We tested for changes in this signature between the brain expression profiles of control, IDR-1018–treated infected, and saline-treated infected mice (Fig. 5B). The ECM transcriptional signature was significantly increased in brains from both saline-treated infected
Hyp1018 regulated gene networks of inflammation, erythropoiesis, cell cycle, and DNA repair. Red genes were up-regulated and green genes were down-regulated in the spleens of IDR-1018–treated compared with saline-treated mice on day 3 of P. berghei ANKA infection. For all networks, differential gene expression was defined as fold change of >1.5 and FDR of <0.1. (A) Network of differentially expressed genes associated with immune responses. (B) Network of differentially expressed genes associated with erythropoiesis. Genes involved in heme biosynthesis are underlined orange. (C) KEGG diagram (47, 48) of the cell cycle, with gene expression values overlaid.
(P = 0.0091) and IDR-1018–treated infected mice (P = 0.0446) relative to uninfected controls. The ECM signature also tended to be higher in saline-treated infected brains than in IDR-1018–treated infected mouse brains, that is, the signature appeared to be suppressed by IDR-1018 treatment (P = 0.0683). There was considerable variation when comparing individual IDR-1018–treated mice to the saline-infected brain group, with brains from mice 1 and 2 showing the greatest differential expression of ECM-associated genes (Fig. 5C). Together with the spleen results (Fig. 5A), these data reveal high transcriptional variability in the IDR-1018–treated infected group.

To elucidate the anti-inflammatory function of IDR-1018 in ECM, we examined the expression of receptors for inflammatory cytokines and chemokines (Fig. 5D and table S2). This gene set, which was significantly up-regulated (P = 0.006) in the saline-infected compared to IDR-1018–treated infected spleens (that is, down-regulated by IDR-1018), but not in the brain. Network analysis of transcriptional changes induced by IDR-1018 in LPS-stimulated human CD14+ monocytes (fig. S2A) revealed targeting of major signaling pathways. These included the nuclear factor κB (NF-κB), mitogen-activated protein kinase, phosphatidylinositol 3-kinase, and Src family kinase pathways and key downstream transcription factors for TNF-α (NF-κB1, NF-κB2, and REL) and IFN-γ signaling (STAT1). Gene set testing of the genes involved in this signaling network in the day 3 ECM expression data (fig. S2B) revealed that IDR-1018 modulated this gene set in a comparable fashion in the context of murine malaria (P = 0.0001 for the comparison of IDR-1018–treated infected and saline-treated infected mice) in the spleen, but not in the brain.

**DISCUSSION**

This study demonstrated the efficacy of adjunctively administered IDR peptide IDR-1018 against ECM. With the aid of advanced bioinformatic analyses, we explored this model for more effective preclinical screening of potential adjunctive therapies for human CM. Immune interventions frequently offer 100% protection against ECM if given prophylactically, but most are not effective against established...
Note that multiple microarray probes can map to the same gene.

weights indicate positive correlation with infection, whereas negative weights indicate negative correlation. Note that multiple microarray probes can map to the same gene.

### Table 1

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infections. A critical evaluation of the *P. berghei* ANKA ECM model resulted in recommendations to improve the model’s utility (2–8): (i) use the ECM model primarily as a preclinical screen for drug development, (ii) focus on adjunctive therapies in combination with anti-malarials, (iii) late-stage rather than early-stage treatment, and (iv) iterative approaches alternating between humans and mice. The current study implemented all recommendations by using IDR-1018 adjunctively with anti-malarial treatment of established infections, iteratively deploying in the mouse model a drug selected for ability to modulate inflammatory responses in human peripheral blood mononuclear cells (PBMCs), and confirming comparable modes of action in murine cells in vitro. The IDR class of peptides is being optimized further in mouse models, and the first IDR peptide has completed phase 1 clinical trials (23).

IDR-1018 treatment suppressed transcriptional pathways of antigen processing, macrophage activity, cell migration, and T cell activation in the spleen, consistent with the known splenic priming of parasite-specific CD8+ and CD4+ T cells contributing to brain pathology in ECM (12, 24, 25). Elucidating the relative importance of these processes in IDR-1018–mediated alleviation of ECM is difficult because ECM results from a complex cellular network (12). We hypothesize that IDR-1018 alleviates brain pathology by down-regulating inflammatory cytokine and chemokine expression, thereby reducing splenic production of highly activated inflammatory T cells and natural killer cells. Proinflammatory cytokines are proposed to mediate pathology in human severe malaria (9, 10), and increased levels of inflammatory cytokines in the plasma and cerebrospinal fluid are strongly associated with severe disease (13, 26), as are promoter polymorphisms in cytokine genes (14).

Transcriptional analyses and in vitro stimulation with TLR agonists showed IDR-1018 reducing production and effects of the proinflammatory cytokines IFN-γ and TNF-α and chemokines CCL3/MIP-1α and CCL4/MIP-1β. These have all been implicated in severe malaria in humans and mice, but no individual molecule is identified as a key regulator in all settings. Neutralization of IFN-γ reduces the incidence of ECM and prevents TNF-α overproduction (27). In humans, heterozygosity for an IFN-γR1 polymorphism was associated with a lowerincidence of CM and malaria fatality (28). TNF-α is proposed as a critical mediator of disease (9, 10), and several studies report associations between TNF-α and CM (29) or severe malaria (30, 31). However, TNF-α–deficient mice remain susceptible to ECM (32), and in a clinical trial, treatment with a monoclonal anti–TNF-α antibody did not protect from CM and exacerbated neurological sequelae (33). Both IFN-γ and TNF-α were also associated with reduced risk of morbidity in longitudinal cohort studies (34, 35).

A role for inflammatory chemokines in severe malaria has only recently been recognized (26), and high levels of MIP-1α and MIP-1β have been linked to human CM (26, 36, 37) and severe malaria in mice (38). Thus, the network and linearized discriminant function analyses used here may represent important tools in the use of *P. berghei* ANKA ECM as a preclinical screen. The ability of a drug candidate such as IDR-1018 to affect a range of functionally related inflammatory mediators increases the likelihood that the effect will translate to human CM.

Transcriptional analyses allowed dissection of ECM development at an extremely early stage, well before mice manifest physical symptoms. The bioinformatic tools ROAST and linear discriminant function analysis enabled discrimination of the transcriptional variability in the IDR-1018–treated group of infected mice. Although changes in gene expression associated with ECM were dampened in all IDR-1018–treated mice, the variations between individual mice could be
associated with differences in survival outcome. Studying coordinate regulation of a linked gene set is far more powerful than examining flux in individual genes. It is possible that future analysis of different time points of infection would further increase precision, allowing tracking of all stages of pathogenesis.

The up-regulation of genes associated with erythropoiesis in the spleens of IDR-1018–treated infected mice correlated with a transient increase in peripheral reticulocyte numbers and enhanced expression of Hbb-b1 in the brain, attributable to increased peripheral erythroblasts. The increase in erythropoiesis may have contributed to the overrepresentation of cell cycle and DNA replication genes. Moderate suppression of erythropoietic transcription in the spleen was observed on day 3 after infection with 10⁶ P. berghei ANKA–infected erythrocytes and was pronounced by day 5 (19), so the effect of IDR-1018 treatment on erythropoiesis could become a counterregulatory force in adjunctive treatment.

In addition to regulating erythropoiesis, erythropoietin (EPO) inhibits the induction of proinflammatory TNF-α and nitric oxide synthase by blocking the activation of NF-κB p65 (39). EPO treatment in the inflammatory disease model of experimental colitis significantly reduces transcription of proinflammatory cytokines and alleviates disease. EPO protects mice from P. berghei ANKA–induced ECM (40, 41). High levels of EPO are also associated with protection from the neurological sequelae of CM in African children (42). Thus, EPO may act independently in a tissue-protective manner in the brains of IDR-1018–treated infected mice and by stimulating erythropoiesis in their spleens.

These data are consistent with other studies where IDR peptides have acted in a multifaceted manner independent of direct antimicrobial activities (15, 16). The broad anti-inflammatory action appears to require rapid systemic or vascular access to PBMCs because IDR-1018 was protective against ECM only when administered intravenously, whereas the anti-inflammatory activity against S. aureus occurred after intraperitoneal injection. Because inflammation is a major cause of morbidity in infections, IDR peptides also offer great promise for adjunctive treatment of infections. Exploring the use of protease-resistant derivatives, such as D-amino acid–containing peptides or peptidomimetics, or liposomally formulated peptides, is expected to increase drug stability and decrease the therapeutically effective dose.

In summary, the focus on adjunctive treatment in combination with first-line antimalarials and the use of advanced bioinformatic tools and network analysis allowed us to use the P. berghei ANKA model of ECM as a preclinical screen to establish the value of an interesting new class of anti-inflammatory drugs.

**MATERIALS AND METHODS**

**Animals and parasites**

Female 8-week-old C57BL/6 and BALB/c mice were purchased from the Animal Resource Centre or the Center for Disease Modeling (University of British Columbia) and maintained under conventional or specific pathogen–free conditions, respectively. Using BALB/c mice as parasite donors, we injected C57BL/6 mice intraperitoneally with 1 × 10⁶ P. berghei ANKA–infected erythrocytes. Animals were checked several times daily for mortality and the development of neurological symptoms indicative of ECM, such as ataxia, loss of reflex, and hemiplegia. All ECM experiments were terminated 14 days after infection. All experiments were approved by the Walter and Eliza Hall Institute Animal Ethics Committee.

**Peptide administration and drug treatment**

The IDR-1018 and IDR-1 peptides were injected either intraperitoneally or intravenously at 8 and 24 mg/kg, respectively, in 200 µl of saline. Control mice were injected with 200 µl of saline. For antiparasitic drug treatment, pyrimethamine (70 mg/liter) (Sigma-Aldrich) and chloroquine (600 mg/liter) (diphosphate salt, Sigma-Aldrich) were administered in drinking water for 7 days. Pyrimethamine was dissolved in

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Fig. 6. IDR-1018 exerted an in vitro anti-inflammatory effect on TLR agonist–stimulated BMDMs. Cells were stimulated with TLR ligands with or without IDR-1018. (A to E) Secreted TNF-α (A, D, and E), MIP-1α (B), and MIP-18 (C) in supernatants at 4, 8, and 24 hours was measured by enzyme-linked immunosorbent assay. BMDMs were stimulated with (A to C) LPS alone or, in combination with IFN-γ, with (D) PAM3CSK4 or (E) CpG 2395. Averages from six wells ± SEM are shown and were representative of three independent experiments. Comparing stimulation with IDR-1018 + TLR agonist (or + TLR agonist + IFN-γ) to the stimulant alone, P < 0.0001 for all 8- and 24-hour time points except TNF-α after 8-hour LPS (P = 0.0014), MIP-1α after 24-hour LPS + IFN-γ (P = 0.0002), TNF-α after 8-hour CpG 2395 (P = 0.0038), and TNF-α after 24-hour CpG 2395 (P = 0.030). (F) Splenocytes from three mice (representative of two experiments) were stimulated with CpG 2395, followed by IDR-1018 and supernatant harvesting at 24 hours. *P < 0.05; **P < 0.01.
dimethyl sulfoxide for a stock solution of 7 mg/ml and diluted 100-fold in water. Chloroquine was dissolved in pyrimethamine-water and adjusted to pH 5 to 6.

Transcriptional signature for ECM
From three published brain microarray data sets from early time points of *P. berghei* ANKA infection in ECM-susceptible mice (19–21), the following data were extracted: From Delahaye et al. (20), we used genes differentially regulated in ECM-susceptible CBA/J and/or C57Bl/6 mice compared to ECM-resistant BALB/c mice (corrected for strain-specific gene expression in uninfected mice) on days 2 and 5 of infection, or on day 5 but not on day 2. From Miu et al. (21), we selected Affymetrix probes differentially expressed in CBA/T6j brains on day 6 of infection compared to uninfected controls. From Sexton et al. (19), we selected genes that were differentially expressed on day 3 of infection in C57Bl/6 mice compared to uninfected controls. Microarray probes were matched across platforms by gene symbol. The combined ECM gene set of 224 up-regulated genes and 59 down-regulated genes is listed in Table S1.

This consensus gene set was used to represent a transcriptional signature of ECM. The directional behavior of this signature was assessed in the microarray expression profiles with rotation gene set testing (ROAST) (22). This approach uses the same linear model framework as the gene-wise analyses in the limma software package. Unsupervised multidimensional scaling plots were created with the plotMDS function of the limma package, with distances defined by the leading 500-fold changes between each pair of samples. Supervised plots discriminating infected from uninfected mice used the regularized linear discriminant function method of the limma package. This function uses an empirical Bayes strategy to avoid indeterminacy of the between-gene covariance matrix when forming the discriminant functions. In both cases, batch effects were removed before plotting by the removeBatchEffects function.

Network analysis
Pathway, Gene Ontology, and network analyses were performed with InnateDB [http://www.innatedb.com (43, 44)] and Ingenuity Pathways Analysis (IPA) [http://www.ingenuity.com] after mapping Illumina probe IDs to National Center for Biotechnology Information (NCBI) RefSeq IDs. Where multiple microarray probes mapped to the same RefSeq ID, the most significant probe was chosen in IPA, whereas InnateDB calculated the average value. In InnateDB, we identified pathways significantly associated with up- and down-regulated genes using the hypergeometric algorithm, and differentially expressed mouse genes were mapped to human Ensembl IDs with InnateDB genes using the hypergeometric algorithm, and differentially expressed mouse genes were mapped to human Ensembl IDs with InnateDB.

BMDM differentiation and stimulation
Mouse BMDMs were prepared by culturing bone marrow cells from C57Bl/6j mice for 7 days in high-glucose Dulbecco’s modified Eagle’s medium with 20% horse serum, 2 mM L-glutamine, 1 mM sodium pyruvate (all from Invitrogen), penicillin (50 U/ml), and streptomycin (50 µg/ml), and supplemented with 30% L-conditioned medium [supernatant of cell line L-929 transfected with a macrophage colony-stimulating factor (M-CSF) expression vector]. On day 7, cells were harvested, washed, and resuspended at 5 × 10⁵ cells/ml in 96-well plates to rest overnight. On day 8, BMDMs were stimulated with or without IDR-1018 (100 µg/ml) with different TLR agonists: LPS (100 ng/ml) (isolated from *Pseudomonas aeruginosa* strain H103), synthetic tripalmitoylated lipopeptide (100 ng/ml) (PAM3CSK4; Invivogen), CpG 2395 (20 µg/ml) (Invivogen), and IFN-γ (10 ng/ml) (ImmunoTools). Splenocyte stimulation was performed comparably except that undifferentiated BALB/c splenocytes and CpG 2395 (10 µg/ml) were used. These experiments were approved by the University of British Columbia Animal Care Committee.

Statistical analyses
The probability of survival for mice with ECM was modeled with logistic regression to test for differences between groups while adjusting for any possible batch effects between independent experiments. One-sided likelihood score tests were conducted with the glm.scoretest function of the StatMod software package (http://www.r-project.org). Because we were only interested in protective outcomes of IDR treatment, one-sided tests were undertaken of the null hypothesis that the probability of survival of mice with ECM was equal or lower after administration of IDR peptides compared to administration of saline against the alternative hypothesis that survival was improved. To check that the asymptotic distributional assumptions of the score tests were satisfactory, we also conducted Fisher-Boschloo-Berger tests of the same hypotheses (46). These gave similar results, so only logistic regression score tests are reported. Parasitemias and reticulocyte counts were compared by unpaired, two-tailed Mann-Whitney tests and cytokine production by unpaired, two-tailed t test with Welch’s correction.

SUPPLEMENTARY MATERIALS
www.sciencetranslationalmedicine.org/cgi/content/full/4/135/135ra64/DC1
Materials and Methods
Fig. S1: IDR-1018 was protective in a mouse model of bacterial infection.
Fig. S2: Key signaling pathways were modulated by IDR-1018.
Table S1: Genes differentially expressed in early ECM.
Table S2: Proinflammatory cytokine and chemokine receptors.

REFERENCES AND NOTES


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