Genetic susceptibility to systemic lupus erythematosus protects against cerebral malaria in mice

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Plasmodium falciparum **has exerted tremendous selective pressure on genes that improve survival in severe malarial infections. Systemic lupus erythematosus (SLE) is an autoimmune disease that is six to eight times more prevalent in women of African descent than in women of European descent. Here we provide evidence that a genetic susceptibility to SLE protects against cerebral malaria. Mice that are prone to SLE because of a deficiency in FcγRIIB or overexpression of Toll-like receptor 7 are protected from death caused by cerebral malaria. Protection appears to be by immune mechanisms that allow SLE-prone mice better to control their overall inflammatory responses to parasite infections. These findings suggest that the high prevalence of SLE in women of African descent living outside of Africa may result from the inheritance of genes that are beneficial in the immune control of cerebral malaria but that, in the absence of malaria, contribute to autoimmune disease.**

Malaria, an infectious disease that kills nearly 1 million children each year in Africa alone, is caused by *Plasmodium falciparum* (*Pf*), the most deadly species of *Plasmodium* that prevails in Africa. The high mortality associated with malaria has exerted an enormous selective pressure on genes that protect against severe malaria even though they may be deleterious in other regards (1). This phenomenon perhaps is best illustrated by the high prevalence in Africa of the hemoglobin S allele (Hb-S). Although Hb-S homozygosity is lethal in children in West Africa (causing sickle cell anemia), Hb-S is maintained at an 18% gene frequency because in the heterozygous state Hb-S confers protection against severe malaria and death (2). *Pf* infections result in a spectrum of diseases ranging from mild, uncomplicated malaria to severe malaria including cerebral malaria and respiratory distress, the primary causes of death from malaria in African children (3). Children in Africa appear to acquire immunity to severe malaria by age 5 y, but immunity to mild malaria requires additional years of repeated exposures (4), suggesting that different immune mechanisms may be at work in controlling severe versus mild malaria. Because severe malaria kills children, it is anticipated that any gene that contributes to immune protection from severe disease would be selected for. Such genes would be beneficial to individuals living in malaria-endemic areas, who frequently are infected with *Pf*. What is not known is whether such genes might have deleterious effects in individuals of African descent who no longer live in a malaria-endemic environment. Although we assume that the genetic selective pressure of malaria would be gender neutral, one of the best examples of the deleterious effects immune selection in Africans may be systemic lupus erythematosus (SLE). SLE is an autoimmune disease that is six to eight times more prevalent in women of African descent living outside Africa than in women of European descent (5), even though little autoimmune disease is reported in Africa (6). The increased risk of SLE in women of African descent correlates with the portion of the genome that is of West African ancestry, indicating a genetic basis for this SLE susceptibility (7). To date, however, the West African genes that account for the high risk of SLE in women of African descent have not been identified (5). Together, these observations suggest that SLE-susceptibility genes may protect against severe malaria but that in the absence of continual exposure to *Pf* such genes may contribute to hyperimmune responses typical of systemic autoimmune disease.

To test directly if SLE susceptibility protects against severe, lethal malaria, we evaluated three mouse strains with defined genetic alterations that have been shown to develop lupus glomerulonephritis with pathology similar to human SLE in a mouse model of cerebral malaria. We analyzed *FcγRIIB*$^{-/-}$*.yaa* mice that are deficient in FcγRIIB, an inhibitory receptor that is central to the control of humoral immune responses (8). FcγRIIB was of particular interest because a human allele that encodes a polymorphism in the transmembrane domain in FcγRIIB that results in a loss of function (9, 10) is significantly more common in Africans (11, 12) and in African Americans (9) than in Europeans and is associated with SLE in Asia (13). Indeed, FcγRIIB deficiency in mice has been shown to reduce the severity of nonfatal *Plasmodium chabaudi* infections that correlated with increased levels of the proinflammatory cytokine TNF-α and with increased antibody levels (11). Willcocks et al. (12) recently provided evidence that a loss-of-function polymorphism in FcγRIIB is associated with protection from severe malaria in African children. In addition to the FcγRIIB deficiency, *FcγRIIB*$^{-/-}$*.yaa* mice have the Y chromosome-linked genetic modifier Yaa, a duplication in the gene that encodes Toll-like receptor 7 (TLR7) (14), a member of one family of the innate immune system's pathogen-associated molecular pattern recognition receptors. We also evaluated mice deficient only in FcγRIIB (*FcγRIIB*$^{-/-}$ mice) (15) and mice with multiple (approximately six or seven) copies of the gene encoding TLR7 (*TLR7.tg* mice) (16). Here we provide evidence that these SLE-prone mice are protected against cerebral malaria. Overall, SLE-prone mice appeared to be protected because of their ability to control their inflammatory responses to parasite infection. These results support the view that the high risk of SLE in women of African descent is related to the protective value of SLE high-risk genotypes in severe malaria.

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Results

Mice with a Genetic Predisposition to SLE Are Protected from Cerebral Malaria. Infection with the ANKA strain of *Plasmodium berghei* (*P. berghei* ANKA) that causes cerebral malaria in susceptible strains of mice is characterized by rapid death, within ≈10 d of infection, at a relatively low parasitemia accompanied by brain microhemorrhages (17). In resistant strains of mice, *P. berghei* ANKA infections result in later death, ≈15 d after infection, caused by high parasitemia and severe anemia without evidence of brain microhemorrhages. At equivalent levels of parasitemia, susceptible strains of mice also show parasitized RBCs in the brain, but resistant mice do not (17).

WT mice, B6 mice, and *FcγRIIB*$^{-/-}$*.yaa* mice (SLE-susceptible mice on the B6 background) were infected with *P. berghei* ANKA at age 10–12 wk. Infected *FcγRIIB*$^{-/-}$*.yaa* mice died significantly later than WT mice ($P < 0.0001$) (Fig. 1*A*). The median survival time was 15 d in 12 *P. berghei* ANKA-infected *FcγRIIB*$^{-/-}$*.yaa* mice and 9.5 d in infected WT mice. No *FcγRIIB*$^{-/-}$*.yaa* mouse survived the infection, as was expected, because even cerebral malaria-resistant mice with *P. berghei* ANKA infections die because of severe anemia. *FcγRIIB*$^{-/-}$*.yaa* and WT mice developed parasitemia at similar rates (Fig. 1*B*), but the WT mice died at significantly lower parasitemias (% parasitized RBC at death = 19 ± 15) than *FcγRIIB*$^{-/-}$*.yaa* mice (% parasitized RBC at death = 42 ± 16) ($P = 0.002$).

After infection, *FcγRIIB*$^{-/-}$*.yaa* animals developed splenomegaly with spleen weights significantly greater than infected WT mice measured at day 7 postinfection (Fig. 1*D*). Spleen size measured at autopsy correlated well with survival times for infected mice (Spearman's $\rho = 0.73$) (Fig. 1*D*). We also measured postmortem weights of brains and kidneys and found that the brains of *FcγRIIB*$^{-/-}$*.yaa* animals were lighter than those of WT mice (Fig. 1*C*) ($P = 0.001$), possibly suggesting less edema. We did not observe significant differences in the weights of kidneys ($P = 0.54$). In contrast to WT mice, all *FcγRIIB*$^{-/-}$*.yaa* animals had developed moderate kidney inflammation (an early sign of SLE) at the time of infection (Fig. S1).

In addition to causing rapid death at relatively low parasitemias, cerebral malaria is characterized by microhemorrhages in the brain (17). Analyses of brain pathology at the time of autopsy showed that the number of microhemorrhages was significantly greater in WT mice than in *FcγRIIB*$^{-/-}$*.yaa* mice (Fig. 2). An example of a microhemorrhage in the brain of an infected WT mouse is shown in Fig. 2, *A* and *B*. Microhemorrhages were quantified and scored on a scale of 0–3 based on the number of microhemorrhages observed in four tissue sections. The scores of WT mice were significantly higher than those of *FcγRIIB*$^{-/-}$*.yaa* mice (Fig. 2*C*), and the brain microhemorrhage scores were inversely correlated with survival (Spearman's $\rho = 0.71$). Collectively, the low brain microhemorrhage scores, prolonged survival, and relatively high parasitemias at death indicate that the *FcγRIIB*$^{-/-}$*.yaa* mice are resistant to cerebral malaria and die of severe anemia and high parasitemia. We also observed that more *FcγRIIB*$^{-/-}$*.yaa* mice than WT mice developed focal meningeal mononuclear infiltrates, although the difference did not reach statistical significance ($P = 0.09$) (Fig. 2 *E–G*). Mononuclear infiltrates into the meninges have been reported in susceptible B6 mice protected from lethal *P. berghei* ANKA cerebral malaria by vaccination with killed blood-stage parasites (18).

The results presented thus far show that *FcγRIIB*$^{-/-}$*.yaa* mice are resistant to cerebral malaria but are unable to control high parasitemia in *P. berghei* infections. We directly evaluated the susceptibility of *FcγRIIB*$^{-/-}$*.yaa* mice to infection with the 17XL strain of *Plasmodium yoelii* (*P. yoelii* 17XL) in which the primary cause of death is severe anemia resulting from high parasitemia, with no cerebral involvement. *FcγRIIB*$^{-/-}$*.yaa* mice had no survival advantage over WT mice in *P. yoelii* 17XL infections (Fig. 3*A*). *FcγRIIB*$^{-/-}$*.yaa* and WT mice died at the same rate, with 100% of animals dying by day 8 after infection with similar parasitemias. *FcγRIIB*$^{-/-}$*.yaa* mice had higher spleen weights than WT mice ($P = 0.03$), but these weights did not correlate with survival (Spearman's $\rho = 0.37$; $P = 0.12$). Brains of *FcγRIIB*$^{-/-}$ animals were lighter than those of WT mice ($P = 0.005$), but kidney weights in the two groups were not different (Fig. S2).

Increased TLR7 Gene Copy Number and FcγRIIb Deficiency Independently Protect Against Cerebral Malaria. To determine if the protection against cerebral malaria observed in *FcγRIIB*$^{-/-}$*.yaa* mice was caused by the absence of FcγRIIB or the increase in TLR7 gene copy number, we determined the survival times for *TLR7.tg* and *FcγRIIB*$^{-/-}$ mice in *P. berghei* ANKA infections. Both *TLR7.tg* and *FcγRIIB*$^{-/-}$ mice develop SLE-like diseases, but disease is somewhat less severe in *TLR7.tg* mice than in *FcγRIIB*$^{-/-}$*.yaa* mice as judged by mean survival time (*FcγRIIB*$^{-/-}$*.yaa*, 4–5 mo; *TLR7.tg*, 7–8 mo; and *FcγRIIB*$^{-/-}$, 8–9 mo). *TLR7.tg* mice tended to survive *P. berghei* ANKA infections longer than control WT mice (Fig. 3*B*): *TLR7.tg* mice had a median survival time of 10 d following infection compared with 7 d for WT control mice. To determine if *TLR7.tg* mice were protected from cerebral disease, the brains were examined at autopsy. The analysis showed microhemorrhages significantly more

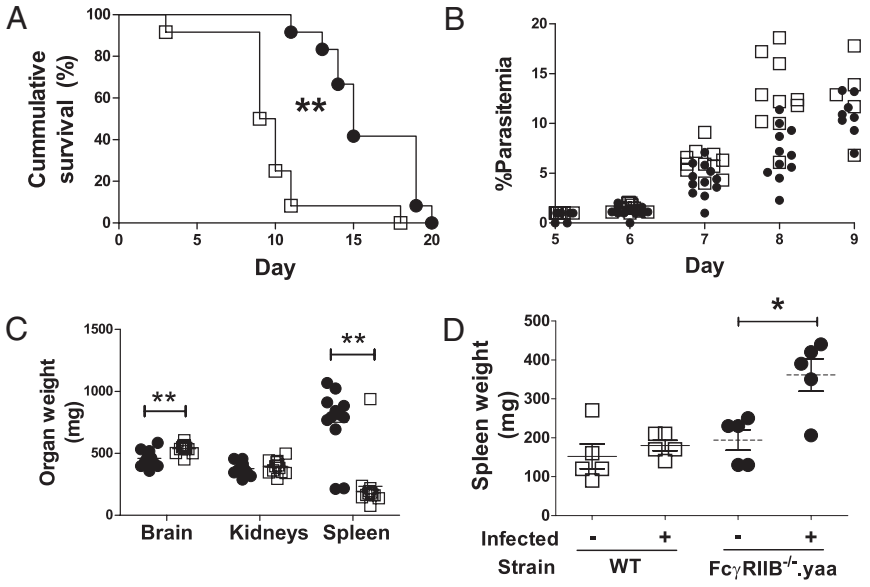

Fig. 1. In *P. berghei* ANKA infections, WT mice die earlier and at lower parasitemias than *FcγRIIB*$^{-/-}$*.yaa* mice. *FcγRIIB*$^{-/-}$*.yaa* ● ($n = 12$) and WT mice □ ($n = 12$) were infected with 1×10^6 *P. berghei* ANKA iRBCs on day 0. (*A*) The percent of *FcγRIIB*$^{-/-}$*.yaa* and WT mice that survived over time is given in Kaplan–Meier curves. Death was defined as parasitemia ≥60%, moribundity, or death ($P < 0.0001$). (*B*) Parasitemias, determined by Giemsa-stained smears of tail blood, are given for *FcγRIIB*$^{-/-}$*.yaa* mice and WT mice over time. (*C*) The weights of brains, kidneys, and spleens of mice determined at autopsy. (*D*) The weights of spleens of *FcγRIIB*$^{-/-}$*.yaa* and WT mice determined at day 7 postinfection. *$P < 0.05$; **$P < 0.01$.

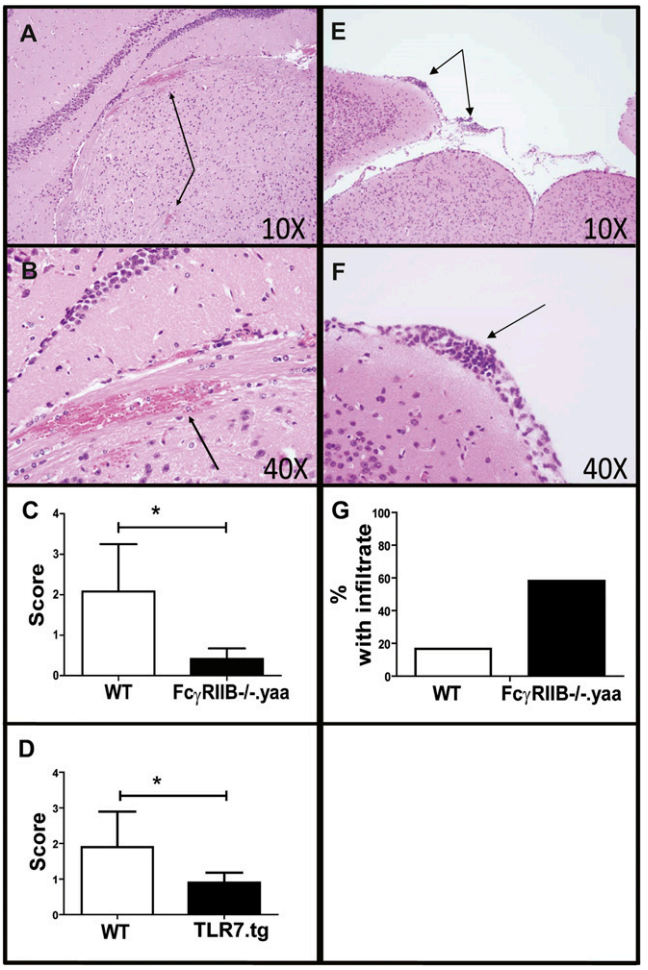

Fig. 2. At death, WT mice have significantly more brain microhemorrhages than *FcγRIIB*$^{-/-}$*.yaa* or *TLR7.tg* mice. (*A* and *B*) Typical brain sections of WT (n = 12) *P. berghei*-infected mice. The arrows in *A* indicate typical microhemorrhages found in WT brains; these microhemorrhages are magnified in *B*. (*C*) Microhemorrhages were quantified, given scores of 0–3 based on the number of hemorrhages in four tissue sections (0 = none; 1 = fewer than five; 2 = 5–10; 3 = more than 10 microhemorrhages). The average scores of *FcγRIIB*$^{-/-}$*.yaa* and WT mice are given (P = 0.04). Shown are the mean and SD. *$P < 0.05$. (*D*) Average microhemorrhage scores for *TLR7.tg* and WT mice (P = 0.046). (*E* and *F*) Typical brain sections of *FcγRIIB*$^{-/-}$*.yaa* mice infected with *P. berghei* (n = 12). Arrows in *E* indicate typical meningeal infiltrates found in *FcγRIIB*$^{-/-}$*.yaa* brains; these infiltrates are magnified in *F*. (*G*) Meningeal infiltrates were evaluated and given a binary score (0 = absent; 1 = present). The percent of WT and *FcγRIIB*$^{-/-}$*.yaa* mice showing focal meningeal infiltrates in brain sections is given.

in WT than in *TLR7.tg* mice (Fig. 2*D*). Taken together with the observation that *TLR7.tg* and WT mice developed parasitemias at similar rates (Fig. 3*B*) but that *TLR7.tg* mice died with significantly higher parasitemias than WT mice (% parasitemia at death 56 ± 8 for *TLR7.tg* mice vs. 28 ± 18 for WT mice; P = 0.001), these data indicate that *TLR7.tg* mice are resistant to cerebral malaria and die of severe anemia caused by high parasitemia. On autopsy, *TLR7.tg* mice also had larger spleens than control mice (P = 0.003), and spleen size correlated well with survival times (Spearman's ρ = 0.79) (Fig. S3). FcγRIIB-deficient mice also survived *P. berghei* ANKA infections longer than WT mice (14 d vs. 7.5 d; P = 0.02) (Fig. 3*C*), had larger spleens than WT mice (P = 0.01) that correlated with survival times (Spearman's ρ = 0.80) (Fig. S3), developed parasitemias at similar rates (Fig. 3*B*) but died with higher parasitemias than WT mice (% parasitemia at death = 63 ± 18 vs. 37 ± 30; P = 0.03). Neither *TLR7.tg* nor *FcγRIIB*$^{-/-}$ mice presented signs of overt autoimmune disease, including kidney inflammation and autoantibodies, at the age they were used in our experiments (8).

Characterization of Immune Responses in *FcγRIIB*$^{-/-}$*.yaa* Mice. At present the immune mechanisms at play in cerebral malaria in either humans or in mouse models are not well understood. In mouse models there is considerable evidence that immune responses to infection, which play a role in the control of parasitemia, also result in strong inflammatory responses and high levels of inflammatory cytokines. The inflammatory response and the sequestration of infected RBCs (iRBCs) in the brain contribute to cerebral malaria (19). We measured a number of cytokines in the peripheral blood of animals before and 5 d after infection with *P. berghei* ANKA to determine if any cytokines were associated with protection against cerebral malaria (Fig. 4 and Table S1). Before infection, only the levels of GM-CSF and IL-10 were significantly different in *FcγRIIB*$^{-/-}$*.yaa* and WT mice (P = 0.002), with *FcγRIIB*$^{-/-}$*.yaa* mice having higher levels of both cytokines (Fig. 4 and Table S1). After infection, however,

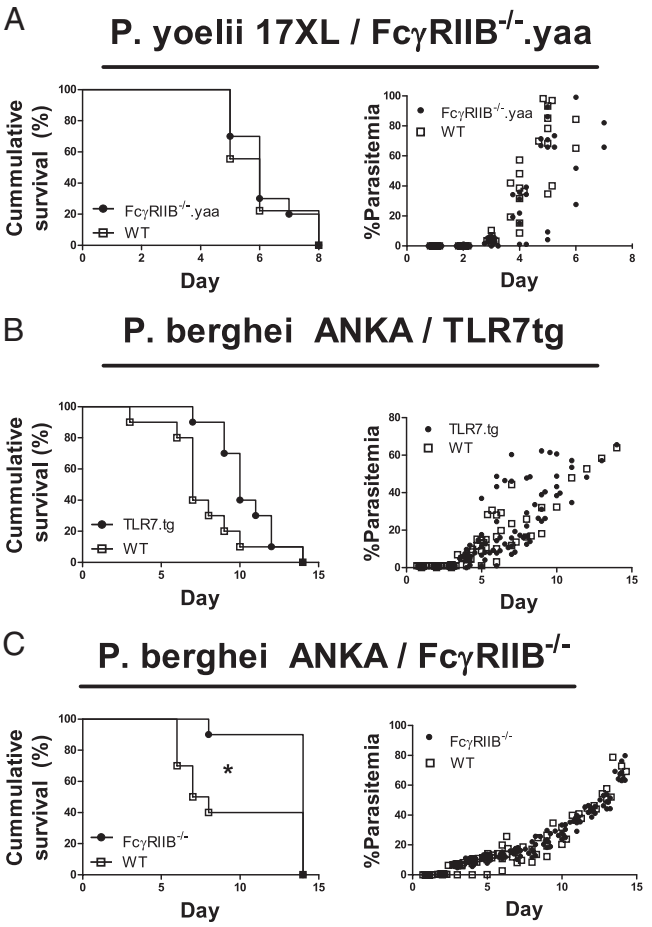

Fig. 3. *FcγRIIB*$^{-/-}$*.yaa* and WT mice are equally susceptible to *P. yoelli XL17* infection, and FcγRIIB-deficiency and TLR7 overexpression independently protect against cerebral malaria. (*A*) *FcγRIIb*$^{-/-}$.yaa (n = 10) and WT mice (n = 9) were infected with 1 × 10^4 *P. yoelii 17 XL* iRBCs. (*Left*) Percent of *FcγRIIB*$^{-/-}$*.yaa* and WT mice that survived over time after infection is shown in Kaplan–Meier curves (P = 0.80). (*Right*) Parasitemias are given with time after infection. (*B*) *TLR7.tg* (n = 10) and WT mice (n = 10) were infected with 1 × 10^6 *P. berghei* ANKA iRBCs. (*Left*) Percent of mice surviving over time is shown in Kaplan–Meier curves (P = 0.07). (*Right*) Parasitemias are given with time after infection. (*C*) *FcγRIIB*$^{-/-}$ and WT mice were infected with 1 × 10^6 *P. berghei* ANKA iRBCs. (*Left*) Percent of *FcγRIIB*$^{-/-}$ and WT mice that survived over time after infection is shown in Kaplan–Meier survival curves (*P = 0.003). (*Right*) Parasitemias with time after infection.

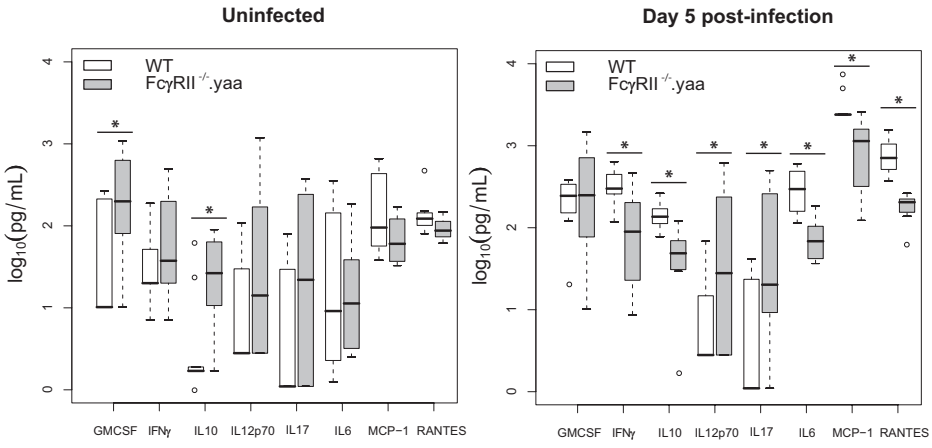

Fig. 4. Cytokine profiles in uninfected and *P. berghei* ANKA-infected *FcγRIIB*$^{-/-}$*.yaa* (n = 10) and WT (n = 10) mice. Serum cytokine levels were measured before infection with *P. berghei* ANKA iRBCs and 5 d postinfection using a multiplex assay. Shown are the log of the concentrations of GM-CSF, INF-γ, IL-10, IL-12p70, IL-17, IL-6, MCP-1, and RANTES in pg/mL Thick lines within the boxes represent the medians of the data, the boxes represent the upper and lower quartiles, dotted lines represent the largest and smallest non-outlier observations, and open dots represent outliers (n = 10) for each group.

the levels of GM-CSF were equivalent in *FcγRIIB*$^{-/-}$*.yaa* and WT mice, and the levels of IL-10 in WT mice rose to levels greater than those in either uninfected or infected *FcγRIIB*$^{-/-}$*.yaa* mice (P = 0.01). After infection WT mice also had significantly higher levels of IFN-γ, IL-12p70, IL-17, IL-6, monocyte chemotactic protein-1 (MCP-1), and regulated upon activation, normal T-cell expressed and secreted (RANTES) than *FcγRIIB*$^{-/-}$*.yaa* mice. It is of interest that after infection *FcγRIIB*$^{-/-}$*.yaa* mice showed pronounced T helper 17 (Th17) skewing as compared with infected WT mice. It may be that the higher levels of the anti-inflammatory cytokine IL-10 before infection and the lower levels of the pro-inflammatory cytokines IL-6, MCP-1, RANTES, and IFN-γ postinfection in *FcγRIIB*$^{-/-}$*.yaa* mice played a role in their protection from cerebral malaria.

To identify alterations in immune cell populations that might correlate with protection from cerebral malaria, we carried out extensive phenotyping by flow cytometry of immune cells in the spleens of *FcγRIIB*$^{-/-}$*.yaa* and WT mice before and 7 d after infection with *P. berghei* ANKA (Fig. 5*A* and Table S2). Most cell subpopulations, including B220^{+}GL7^{+} germinal center B cells, B220^{int}CD138^{+} plasma cells, B220^{+}CD69^{+} activated B cells, CD4^{+}CD69^{+} activated helper T cells, and CD8^{+}CD69^{+} activated cytotoxic T cells, were increased in *FcγRIIB*$^{-/-}$*.yaa* mice as compared with WT mice before infection but after infection increased to similar levels in both infected WT and *FcγRIIB*$^{-/-}$*.yaa* mice. However, there were fewer natural killer cells in infected *FcγRIIB*$^{-/-}$*.yaa* mice than in infected WT mice, and infection reduced the percent of forkhead box P3 (FoxP3)-positive regulatory T cells (Tregs) in WT mice but had no effect in *FcγRIIB*$^{-/-}$*.yaa* mice. The pattern of expression of inducible costimulator (ICOS) was of interest (Fig. 5*B*). Strikingly, most CD4^{+} T cells from uninfected *FcγRIIB*$^{-/-}$*.yaa* animals expressed ICOS at intermediate levels that usually correlate with a response to IL-10 (20) (Fig. 5*B*), consistent with the observed increase in IL-10 levels in *FcγRIIB*$^{-/-}$*.yaa* mice before infection. Infection of *FcγRIIB*$^{-/-}$*.yaa* mice had little effect on ICOS expression (Fig. 5*B*), with the majority of T cells continuing to express intermediate levels of ICOS. In contrast, T cells in uninfected WT mice did not express ICOS, but infection resulted in an increase in ICOS expression to high levels in 50% of the WT CD4^{+} T cells. ICOS^{high} T cells synthesize IL-2, IL-3, and IFN-γ (20), consistent with the observation that infected WT mice produce more IFN-γ than do uninfected mice. Similarly the small number of ICOS^{high} T cells in infected *FcγRIIB*$^{-/-}$*.yaa* mice may explain why those mice produced less IFN-γ than infected WT mice. Although uninfected *FcγRIIB*$^{-/-}$*.yaa* mice had significantly more CD4^{+}CD44^{+} activated T cells than did WT mice, infection resulted in an increase in CD4^{+}CD44^{+} activated T cells in both *FcγRIIB*$^{-/-}$*.yaa* and WT mice (Fig. 5*B*). Collectively, the characteristics of the splenic immune cell populations suggest that cells from *FcγRIIB*$^{-/-}$*.yaa* mice had features of a controlled chronic activation before infection that were absent in WT mice and that infection of *FcγRIIB*$^{-/-}$*.yaa* mice resulted in reduced inflammatory responses as compared with WT mice.

The lower levels of inflammatory cytokines, particularly MCP-1 and RANTES, observed in the peripheral blood of infected *FcγRIIB*$^{-/-}$*.yaa* as compared with WT mice could indicate reduced recruitment of inflammatory and cytotoxic immune cells to the brain and might explain the reduced hemorrhagic pathology of *FcγRIIB*$^{-/-}$*.yaa* mice. We examined the immune cell populations in the brains of WT and *FcγRIIB*$^{-/-}$*.yaa* mice before and 7 d after infection with *P. berghei* ANKA. We observed that CD4^{+} and CD8^{+} T cells were recruited to the brains of both WT and *FcγRIIB*$^{-/-}$*.yaa* infected mice (Fig. 6*A*). To determine whether the T cells present in the brains of WT and *FcγRIIB*$^{-/-}$*.yaa* mice were differentially activated, we measured the mRNA levels of granzyme B, IL-10, IFN-γ, IL-6, and IL-12 in the brains of these mice before and after infection. We found no significant differential expression of these mRNAs in WT and *FcγRIIB*$^{-/-}$.

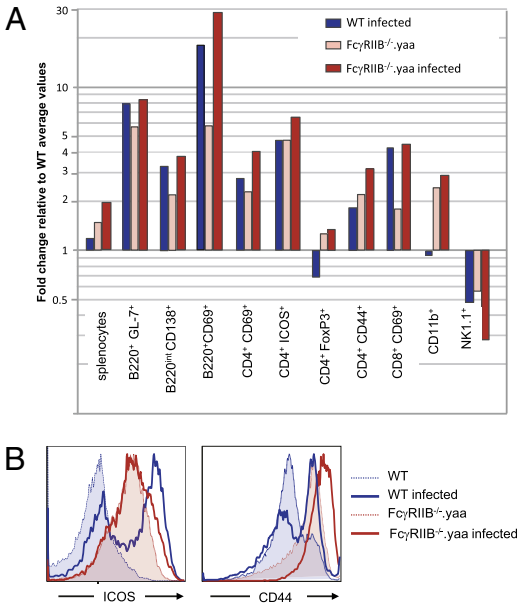

Fig. 5. Alterations in spleen cell phenotypes related to protection from cerebral malaria. (*A*) Immune cell populations were analyzed by flow cytometry. Average values of three mice per group are shown. Values are given relative to the number of splenocytes in uninfected WT mice or the percentage in WT mice of each subpopulation Absolute cell numbers are provided in Table S2. (*B*) The histograms show fluorescence intensity for ICOS (*Left*) and CD44 (*Right*) gating on CD4^{+} T cells. One representative experiment of six is shown.

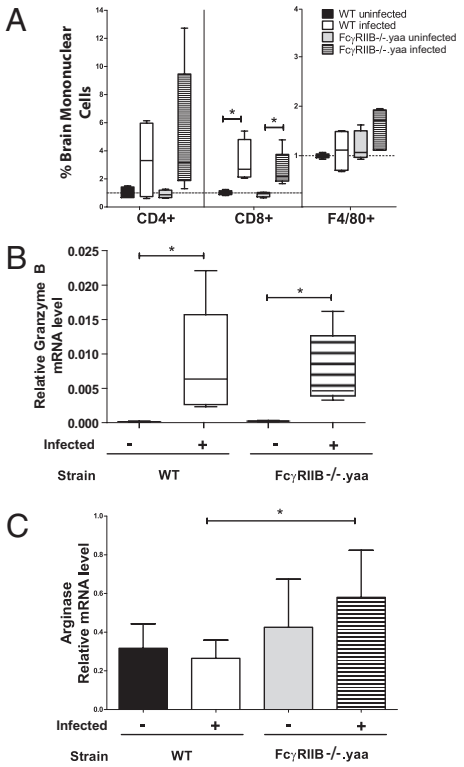

Fig. 6. Recruitment of immune cells to the brains of infected mice. (*A*) Mononuclear cells isolated from brains of uninfected (n = 5) and infected (n = 4) WT and uninfected (n = 5) and infected (n = 5) *FcγRIIB*$^{-/-}$*.yaa* mice were characterized by flow cytometry. Shown are the percent of live mononuclear brain cells that were CD4$^+$ T cells, CD8$^+$ T cells, or F4/80$^+$ macrophages. Boxes show the median and 25th and 75th percentiles; whiskers show the largest and smallest values. *$P < 0.05$. (*B*) Granzyme B mRNA expression was measured in brain samples of infected (n = 5) and uninfected (n = 2) WT mice and in infected (n = 4) and uninfected (n = 3) FcγRIIB$^{-/-}$.yaa mice. (*C*) Arginase mRNA expression was measured in brain samples of infected (n = 5) and uninfected (n = 2) WT mice and in infected (n = 4) and uninfected (n = 3) FcγRIIB$^{-/-}$.yaa mice. For *B* and *C*, expression of the L32 housekeeping gene was used as standard in the calculations. Shown are means and SD. *$P < 0.05$.

yaa mice and provide the results for granzyme B to illustrate this point (Fig. 6). Granzyme B levels are low in the brain of uninfected WT and *FcγRIIB*$^{-/-}$*.yaa* mice and rise to similar levels in malaria-infected mice. Thus, although similar numbers of T cells in similar activation states are recruited to the brains of WT and *FcγRIIB*$^{-/-}$*.yaa* mice, cerebral disease is controlled only in the latter.

The number of F4/80$^+$ macrophages in the brain did not increase appreciably in infected WT animals but was somewhat increased in *FcγRIIB*$^{-/-}$*.yaa* mice both before and after infection. It has been reported that M2 macrophages can decrease T cell-mediated central nervous system disease (21), causing an increase in the number of Tregs and increases in both IL-10 and GM-CSF, characteristics that we observed in *FcγRIIB*$^{-/-}$*.yaa* mice before infection (Fig. 4). M2 macrophages are characterized by high levels of arginase mRNA (22). We detected M2 macrophages in the brains of *FcγRIIB*$^{-/-}$*.yaa* mice at autopsy and found a twofold increase in arginase mRNA in both infected and uninfected *FcγRIIB*$^{-/-}$*.yaa* mice relative to WT mice (Fig. 6*B*), implicating M2 macrophages in the protection of *FcγRIIB*$^{-/-}$*.yaa* mice from cerebral malaria.

Discussion

Here we provide evidence that genetic alterations that are responsible for the development of an SLE-like autoimmune disease in mice, namely an FcγRIIB-deficiency and TLR7 duplication, are protective against cerebral malaria. *FcγRIIB*$^{-/-}$ is a potent SLE-susceptibility gene capable of interacting with a variety of other loci to modify the initiation and progression of autoimmune disease (8). A single nucleotide polymorphism in the gene encoding the human FcγRIIB that abrogates receptor function is strongly associated with susceptibility to SLE in both Caucasians and Southeast Asians (13). Willcocks et al. (12) recently reported that the minor allele of this polymorphism is more common in Southeast Asians and Africans than in Caucasians and that homozygosity for the minor allele is associated with substantial protection from severe malaria in children in Kenya (12), although this study was not powered to detect associations with a specific type of severe malaria, for example cerebral malaria versus severe malaria anemia. Consistent with the observation by Willcocks et al., it was shown earlier that macrophages from individuals homozygous for this polymorphism efficiently engulfed *Pf* trophozoites (11). Collectively, these findings and those reported here may help explain the high prevalence of SLE among individuals of African descent living outside Africa.

Despite the prevalence of SLE in women of African descent, current evidence indicates that there is little autoimmunity in Africa (6, 23). Indeed, SLE is highly prevalent only in Africans living outside *Pf*-endemic areas. These observations suggest that chronic reinfection with *Pf*, as occurs in Africa, may attenuate the potential of SLE high-risk genes to cause autoimmunity. If this speculation is correct, it would be predicted that malaria infections attenuate autoimmunity. Using the spontaneous autoimmune disease models of New Zealand Black (NZB) and (NZB × New Zealand White) F1 hybrid mice and the nonlethal mouse strains of *P. yoelii*, Greenwood et al. (24) provided evidence that malaria infections have some protective effects against the development of autoimmune disease. Thus, malaria may suppress autoimmunity in individuals with SLE-susceptibility alleles. It will be of interest to determine what features of malaria parasite infections are responsible for attenuating autoimmune responses.

It also will be of considerable interest to understand the cellular and molecular basis of the observed protection of SLE-prone mice from cerebral malaria. In mice, cerebral malaria is characterized by a complex cascade of events including breakdown of the blood–brain barrier, sequestration of iRBCs in the brain microvessels, accumulation of leukocytes in the brain, and the production of proinflammatory cytokines (25, 26). The analyses of a variety of mice deficient in cytokines (27, 28), chemokines and chemokine receptors (29–31), and TLRs (32) have implicated a variety of immune mechanisms in resistance to cerebral malaria but have not led to a clear picture of the underlying cause of disease pathology. Although the genetic alterations in the SLE-prone mice described here are well defined, the cellular and molecular mechanism by which these alterations result in autoimmunity is not fully understood. The immune parameter data provided here suggest that *FcγRIIB*$^{-/-}$*.yaa* mice develop chronic inflammation with a Th17 skewing and a concomitant induction of regulatory pathways that reduce the highly Th1-biased inflammatory responses in cerebral malaria. We speculate that *FcγRIIB*$^{-/-}$*.yaa* mice may be protected from cerebral malaria by decreasing the overall inflammatory response to the parasite. The feedback regulatory pathway that reduces acute inflammatory responses in *FcγRIIB*$^{-/-}$*.yaa* mice could involve IL-10, because it has been shown that this pathway provides a feedback control of autoimmune responses (33). Regulation also could involve the M2 macrophages that we detected in greater numbers in the brains of *FcγRIIB*$^{-/-}$*.yaa* mice. Indeed, M2 macrophages have been shown to attenuate T cell-mediated brain disease in experimental autoimmune encephalomyelitis (21).

In conclusion, the results presented here provide evidence for a relationship between SLE high-risk alleles and protection against cerebral malaria. At present, the association of genes with SLE susceptibility in women of African descent remains largely unexplored. Once identified, such genes could be tested for their protective value against cerebral malaria in African

children. Future progress in deciphering the cellular and molecular mechanisms underlying the relationships between host susceptibility to autoimmunity and protection from cerebral malaria may lead to new strategies for therapeutics both for SLE and for cerebral malaria.

Materials and Methods

All experiments were approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee.

Animals. Male C57BL/6 mice (10- to 12-wk-old) were obtained from Jackson Laboratories. *FcγRIIB*$^{-/-}$*.yaa*, *FcγRIIB*$^{-/-}$, and *TLR7.tg* mice were bred at the Laboratory of Immunogenetics in the National Institute of Allergy and Infectious Diseases animal facility.

Malaria Infections. Mice were infected with *P. berghei* ANKA or *P. yoelii* 17XL by injecting i.p. 1×10^6 *P. berghei* ANKA or 1×10^4 *P. yoelii* 17XL iRBCs obtained from C57BL/6 or BALB/c mice, respectively. Parasitemias in infected mice were quantified by examining Giemsa-stained blood smears.

Cytokine Measurements. Blood was collected on days 0, 3, and 5 postinfection, and sera were stored at −80 °C until analyzed for IL-1–6, IL-10, IL-12p70, IL-17, macrophage inflammatory protein 1α (MIP-1α), MCP-1, RANTES, GM-CSF, IFN-γ, and TNF-α using the Q-Plex Human Citokyne kit (Quansys Biosciences) according to the manufacturer's instructions.

Real-Time PCR. Cells were lysed in TRIzol (Invitrogen), and total RNA was isolated using the RNeasy Mini Kit (QIAGEN). One microgram of total RNA was reverse transcribed using the iScript cDNA Synthesis Kit (Bio-Rad). Quantitative PCR was performed using the real-time iCycler PCR platform (Bio-Rad). The sequences of primers for arginase 1 (Arg1) were described previously (34). Arg1 mRNA levels were normalized to that of L32 ribosomal RNA. PCR primers for L32 were 5′-AGAGGACCAAGAAGTTCATCAGGC-3′ and 5′-CTCCTTGACATTGTGGACCAGGAA-3′.

Pathology. Animals that reached 60% parasitemia, that became moribund, or that were found dead were dissected, and kidneys, cerebrum, and spleens were fixed in 10% neutral buffered formalin. The tissues were weighed, sectioned, and stained with hematoxylin-eosin. The tissues were evaluated by pathologists blinded to the experimental design. Four tissue sections per animal were analyzed, and brain hemorrhages were scored on a scale from 0 to 3 where 0 represented no hemorrhages, 1 represented >5 hemorrhages, 2 represented 5–10 hemorrhages, and 3 represented >10 hemorrhages. The degree of kidney inflammation was scored from 0 to 3 where 0 represents no inflammation, 1 represents mild inflammation, 2 represents moderate inflammation, and 3 represents severe inflammation. Meningeal infiltrates were evaluated on a binary scale in which 0 indicated absent, and 1 indicated present.

Flow Cytometry Analysis. Single-cell suspensions from spleens were prepared from infected and uninfected *FcγRIIB*$^{-/-}$*.yaa* and WT mice. Mononuclear cells were isolated from the brains of mice infected with malaria on day 7 postinfection. The brains were homogenized, and mononuclear cells were isolated using 40–70% discontinuous Percoll gradients (35). Total cell numbers were determined by counting on a hemocytometer and were analyzed by FACS. Antibodies to the following antigens were used for FACS analysis: B220, CD4, CD8, CD11b, GL7, Fas, CD21, CD23, CD25, CD69, CD44, CD45RB, CD62L, NK1.1, and ICOS (BD Pharmingen). For forkhead box P3 (FoxP3) staining, an anti-mouse FoxP3 staining kit from Ebioscience, was used. Samples were analyzed on a FACSCalibur flow cytometer (BD Biosciences), and data were analyzed using FlowJo software (Tree Star Technologies).

Statistical Analysis. A detailed description of the statistical methods is provided in *SI Materials and Methods*.

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