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miR-146a and miR-155 Delineate a MicroRNA Fingerprint Associated with Toxoplasma Persistence in the Host Brain

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SUMMARY

microRNAs were recently found to be regulators of the host response to infection by apicomplexan parasites. In this study, we identified two immunomodulatory microRNAs, miR-146a and miR-155, that were coinduced in the brains of mice challenged with Toxoplasma in a strain-specific manner. These microRNAs define a characteristic fingerprint for infection by type II strains, which are the most prevalent cause of human toxoplasmosis in Europe and North America. Using forward genetics, we showed that strain-specific differences in miR-146a modulation were in part mediated by the rhoptry kinase, ROP16. Remarkably, we found that miR-146a deficiency led to better control of parasite burden in the gut and most likely of early parasite dissemination in the brain tissue, resulting in the long-term survival of mice.

INTRODUCTION

Toxoplasma gondii is a widespread obligate intracellular protozoan parasite causing toxoplasmosis, a potentially severe disease in immunocompromised or congenitally infected humans. Central to transmission and pathogenesis is the ability to traffic to the CNS following host infection and to undergo transformation from the rapidly dividing, disease-causing tachyzoite to a long-lived, slow-dividing bradyzoite contained within tissue cysts. This bradyzoite cyst sustains establishment of chronic infection. Bradyzoites can spontaneously reconvert back to tachyzoites, but their dissemination is effectively prevented by the immune system. Upon host cell invasion, Toxoplasma parasites grow inside a parasitophorous vacuole (PV), a dynamic niche shaped by the tachyzoite to support its growth. Host stromal and immune cells respond to infection with profound transcriptional changes, including genes involved in mounting Th1-type immune response to infection (Jensen et al., 2011; Hunter and Sibley, 2012). Several strain-specific parasite effectors are secreted into the host cells where they neutralize cell autonomous immune defenses or subvert gene expression (Howard et al., 2011; Hunter and Sibley, 2012). Many known secreted molecules are either released from rhoptry secretory organelles early in host cell invasion (e.g., ROP16, Saeij et al., 2007) or are released from dense granules (e.g., GRA24, Braun et al., 2013).

In addition, apicomplexan parasites may also interfere with host miRNA populations in a parasite-specific manner, suggesting this RNA silencing pathway as a way of reshaping cellular environment (Hakimi and Cannella, 2011). Previous work also suggests that miRNA-based regulatory pathways may contribute to the mounting of host cell responses/defenses (e.g., the inflammatory response) (Ding and Voinnet, 2007).

The present study confirms that Toxoplasma alters the host cell miRNA profile through a mechanism that requires live parasites that are able to invade and divide (Zeiner et al., 2010). Notably, we now describe significant and exclusive upregulation by cystogenic strains (type II) of miR-146a expression, a key inflammatory response regulator (Taganov et al., 2006). Using forward genetics, we show that strain-specific differences in miR-146a expression modulation are controlled by the parasite-secreted kinase ROP16. Furthermore, miR-146a ablation affects early parasite burden, leading to significant differences in interferon (IFN)-γ production and better survival in normally susceptible C57BL/6 mice. Last, mice challenged with type II strains had higher brain levels of miR-146a but also of miR-155, another immunoregulatory RNA (Lindsay, 2008). Collectively, these data uncover a microRNA signature that typifies Toxoplasma long-term persistence and latency in the host brain.
RESULTS

Specific Alteration of Host Cell miRNA Profile by Toxoplasma Infection

In this study, we profiled the steady-state levels of cellular mature miRNAs from primary human foreskin fibroblasts (HFF) infected with the virulent type I (RH) strain (Figure S1A; Table S1). Overall, our data mirror those published previously showing that Toxoplasma alters the levels of ~5%–10% of host miRNAs in tachyzoites-infected human fibroblasts (Zeiner et al., 2010). We compared host miRNA expression levels in cells infected with Toxoplasma, Theileria, or Cryptosporidium using our data set and previously published data sets, and this revealed that these apicomplexan parasites modulate the expression of a restricted panel of miRNAs in a specific manner, most likely in order to suit their respective biology. This can be illustrated by miR-155, a microRNA implicated in immune response (Lindsay, 2008) that was strongly induced by both Toxoplasma (Figures S1B and Theileria (Marsolier et al., 2013) while being unaffected by Cryptosporidium infection (Zhou et al., 2009).

Patterns of expression of host microRNA were linked to the time course of infection and multiplicity of infection (MOI) (Figure S1C; data not shown). Although the levels of miR-1, -26a, -29a, and -221 marginally decreased 6 hr postinvasion and showed a clear inhibition at 15 and 24 hr, miR-1246 increased rapidly at 6 hr and remained high thereafter (Figure S1C). Control cells exposed to heat-killed tachyzoites displayed a miRNA expression profile similar to that of uninfected control samples indicating that interference with the host RNA silencing machinery required live parasites, still able to invade and divide, rather than through signaling molecules diffusing in the cell-culture media (Figure S1C; data not shown). Overall, these changes are a specific response to Toxoplasma infection because the levels of the aforementioned miRNAs remain unchanged in cells infected with Neospora caninum, a close relative of Toxoplasma (Figure S1C).

Toxoplasma Infection Modulates Host miRNAs Biogenesis at Multiple Levels

Theoretically, interference with the miRNA pathway could occur at the levels of miRNA transcription, biogenesis, or activity (Hakimi and Cannella, 2011). In this respect, the inhibition of miR-1, -26a, and -29a may occur at the transcriptional level because the level of their precursors (pre-miRNA) decreased simultaneously postinvasion (Figure S1C). A common feature of these three microRNAs is that they are classified as repressed by the oncogenic transcription factor c-Myc (Chang et al., 2008). It appears that they belong to a larger panel of miRNAs defined as core Myc-regulated genes (~20 miRNAs) that are effectively repressed 6 hr postinvasion by Toxoplasma (Figure S2A). This is consistent with the induction of the Myc protein by Toxoplasma (Figure S2B). Although acting at the transcriptional level, Toxoplasma infection also potentiates the Dicer-mediated processing of host cell miRNAs. For instance, the mature miR-1246 drastically increased with the expected decrease of its precursor, suggesting that infection by live Toxoplasma tachyzoites affects dicer processing (Figure S1C). This response reminds us of the effect of the RNA binding protein Lin-28 on the processing of the microRNA Let-7 (Rybak et al., 2008).

Toxoplasma Upregulates miR-146a Levels in a Strain-Specific Manner

We next considered whether altered miRNA levels were relevant to differences in pathogenesis among strain types. We compared the levels of cellular microRNAs in human fibroblasts infected with type I (RH) strain, which normally does not make tissue cysts, to the cystogenic type II (ME49) strain (Figure 1A; Table S2). Among the miRNAs differentially expressed across strains, miR-146a was substantially and significantly induced by ME49 tachyzoites, whereas the RH parasites did not affect its expression (Figures 1A, 1B, and S2C). The miR-146a miRNA family has two evolutionary conserved genes: miR-146a and miR-146b, which are located on separate chromosomes, in quite unrelated sequence contexts, but differ in their mature sequence by only two nucleotides at the 3′ end (Figure S3A) (Taganov et al., 2006). We next assessed the kinetics of both miR-146a and miR-146b to discriminate between miR-146 family paralogs in human and murine cells infected with Toxoplasma strains of the three main clonal lineages (Figures 1C and 1D). Twenty-four hours postinvasion, miR-146a was dramatically induced (~30- to 60-fold) by type II (PRU and ME49) strains compared to human fibroblasts infected with type I (RH and GT1) or type III (CTG) strains (Figures 1C), miR-146a upregulation by ME49 started 6 hr postinvasion and was most dramatic after 24 hr (Figure 1D). This regulation is likely to be at the level of transcription because a similar upregulation was observed at the level of the primary miR-146a transcripts (pri-miR-146a) in cells infected with type II parasites (Figure 1E). Additionally, the levels of miR-146a increased slightly but exclusively in response to infection by a type II strain in human embryonic kidney 293 and astrocytoma U373 cells, reflecting the robustness of this phenotype using different host cell types (Figures S2D and S2E). Whereas the amounts of mature miR-146a were increased by Toxoplasma infection, miR-146b levels remained unchanged (Figure 1D), suggesting that this miRNA family has a rather specific mode of regulation of expression involving more than one point of control.

miR-146a is widely expressed throughout the hematopoietic system (Boldin et al., 2011). Its expression is initially low in precursor cells and increases with maturation and activation, typically in response to Toll-like receptor (TLR) agonists. In line with previous findings, we found that the levels of miR-146a in murine bone marrow-derived macrophages (BMDMs) and in human primary astrocytes were significantly higher when compared to fibroblasts (Figure S2F; data not shown). However, Toxoplasma did not alter miR-146a expression in BMDMs, nor in astrocytes, most likely because the maximum threshold limit was already reached in these cells. In summary, miR-146a expression was distinctively upregulated in a wide range of nonhematopoietic cells following infection by type II strains of Toxoplasma.

Toxoplasma Type II Strains Induce miR-146a, an NF-κB-Dependent Gene, in a GRA15-Independent Fashion

miR-146a was initially found to be a NF-κB-dependent gene (Figure S3A; Taganov et al., 2006). This response is consistent with the strain-specific activation of the host NF-κB pathway by Toxoplasma (Rosowski et al., 2011). Indeed, type II tachyzoites, unlike types I and III, activate the nuclear translocation of NF-κB p65
RelA promoting its transcriptional activity, which may account for the type II-dependent induction of miR-146a. The dense granule protein GRA15 was reported as sufficient to mediate the effect as it intersects the NF-κB pathway downstream of MyD88, interleukin (IL)-1R, and TRIF but upstream of TRAF6 and the IKK complex (Rosowski et al., 2011). We therefore asked whether GRA15 might be involved in miR-146a induction by Toxoplasma. However, GRA15 deletion in the type II (Pru A7) did not significantly limit miR-146a induction in the infected fibroblast cells (Figure S3B). Similarly, ectopic expression of a type II allele of GRA15 in a type I strain (Figure S3C), while activating NF-κB p65 (Rosowski et al., 2011; data not shown), did not alter miR-146a levels (Figure S3D). Therefore, miR-146a induction by Toxoplasma is independent of GRA15. However, the fact that GRA15 has an effect on some NF-κB family members (e.g., p65 subunit) but not on others (Rosowski et al., 2011) indicates that alternative parasite-derived factors other than GRA15 are likely involved in NF-κB-dependent (p65-independent) miR-146a regulation.

Genome-wide Scan of the miR-146a Phenotype Reveals a Single Major QTL

To further assess if the increase of miR-146a levels in cells infected with Toxoplasma takes place via an active mechanism...
controlled by a parasite-derived product, we took advantage of the aforementioned strain-specific difference to identify the responsible gene(s). If the strain-specific regulation of miR-146a has a genetic basis, it should accordingly segregate among F1 progeny derived from a cross between two strains (II versus I or III) that differ in its regulation. We therefore monitored miR-146a levels in fibroblasts infected separately with 27 F1 progeny derived from the II × III cross (Behnke et al., 2011). As expected, the parental type II (ME49-FUDR R) strain induced significantly higher miR-146a levels compared to the type I (GT1-SNF R) strain, and among the recombinant progeny a range of distinct phenotypes was observed (Figure 2A). Neither in vitro growth (data not shown) nor virulence reported previously (Behnke et al., 2011) were correlated with altered expression of miR-146a among the progeny (Figure 2B). A genome-wide scan for association of Toxoplasma genetic markers and the expression level of miR-146a identified a single high-probability quantitative trait loci (QTL) on chromosome VIIb with a high logarithm of odds score (LOD = 10, Figure 2C) that was associated with >92% of the inherited variation. Our data allowed us to restrict the locus involved to a region between the genetic markers 55.m05046_at5 and 55.m010299_at3, representing a maximum size of 1.2 Mb spanning 200 predicted protein encoding genes (Figure 2B). Unexpectedly, we identified the Toxoplasma polymorphic ROP16 kinase as the most likely candidate (Figure 2B). ROP16 is a specialized phosphotyrosine kinase that is released from the rhoptries and injected into the host cell nucleus to activate STAT3/6 signaling pathways (Saeij et al., 2007). To strengthen our genetic analysis, we repeated the genome-wide scan using type II × III recombinant progeny (Khan et al., 2005) and validated the QTL containing ROP16 for the miR-146a phenotype (Figure S4). In addition, both genetic screens...
confirmed that the type-II-dependent miR-146a induction was clearly GRA15 independent (Figures 2B and S4B).

**ROP16 Suppresses miR-146a Expression in a Strain-Specific Fashion**

To assess whether ROP16 was directly involved in the strain-specific activation of miR-146a, we deleted the gene in both RHΔku80 (type I) and PruΔku80 (type II) strains (data not shown). In line with previous reports (Saeij et al., 2007), we showed that with parasites deleted for ROP16 in type I (RHΔku80Δrop16) the sustained STAT3/6 tyrosine phosphorylation was no longer observed (Figure 3A; data not shown). Deletion of ROP16 also significantly enhanced the induction of miR-146a mediated by Toxoplasma (Figure 3B). In marked contrast, ROP16 deletion in type II (PruΔku80Δrop16) strain did not alter miR-146a levels (Figure 3C). Taken together, these data suggest that the kinase acts as a repressor of the expression of this specific miRNA.

miR-146a and miR-155 Are Highly Induced in Mice Brain Chronically Infected with Type II Strains

Because the induction of miR-146a in stromal cells was restricted to infection by type II strains, which exhibit low virulence and are cystogenic in mice, we further assessed whether this microRNA might play a critical role in chronic toxoplasmosis. Accordingly, we monitored the levels of eight microRNAs that were upregulated (miR-155 and miR-146a), downregulated (miR-150, miR-101, let-7b, miR-29a, and miR-125b), or unchanged (miR-146b and miR-132) following Toxoplasma infection of stromal cells (Figures 1A and S1A). We assessed miRNA levels in the brain of mice infected with a dose of parasites that allows survival through acute infection (<14 days) and establishment of chronic infection (Figure 4A). Surprisingly, miR-146a and miR-155 levels increased.

In this respect, ectopic expression of an additional type I allele of ROP16 in the type II (Pru) strain (ROP16-I, Saeij et al., 2007) downregulated the level of miR-146a by 2-fold (Figure 3D). These data confirm that the type I allele of ROP16 suppresses miR-146a expression, whereas the type II allele lacks this activity. Although ROP16 is released from the rhoptries in the early time of infection (1–6 hr postinfection, Saeij et al., 2007), it has a marked repressive effect on miR-146a beyond 15 hr of infection (Figure 3E), which is consistent with the ability of the kinase to sustain phosphorylation of STAT3/6 in the late phase of infection (Saeij et al., 2007).
in the brain of chronically infected mice in a sustained fashion (6–10 weeks postinfection [p.i.]), regardless of the genetic background of the mouse host (i.e., C57BL/6, BALB/c or Swiss) or the type II parasite used (i.e., Pru A7, Pru ku80, or ME49) (Figures 4A, S5A, and S5B). This miRNA signature is not a broad response to the mere presence of parasites in the brain (detected 11 days p.i.), but rather correlates well with the presence of fully developed cysts in the brain (>20 days p.i., Figure 4B) that persist throughout the life of the chronically infected mice (up to 10 weeks p.i.) (Figure 4A). In contrast, the relative levels of miR-155 and miR-146a are distinctly low in the brains of mice chronically infected with the mouse-avirulent type III (CTG) strain (Figure 4C); yet, this strain causes nonlethal chronic latent infection characterized by a proper positive serology (Figure 4D). The main difference with ME49 infection was that mice chronically infected with CTG had a low number of cysts in their brain (Figure 4E), suggesting a likely relationship between cyst burden and the alteration of miR-155 and miR-146a levels in the mice brain (Figures 4C and 4E). In this fashion, the high levels of miR-146a in mouse brains with high cyst burden are consistent with the type II strain-restricted regulation of the microRNA in stromal cells. Surprisingly, miR-155, while being induced in the brain only when infected by type II strains (Figures 4A, S5A, and S5B), has a strain-independent expression profile in stromal (Figure 1C).
and phagocytic cells infected in vitro (Figure S2F). This suggests that the miRNA fingerprint that marks cystogenesis in chronically infected mice has multiple strain-dependent and -independent determinants.

miR-146a belongs to a large family of circulating microRNA that cofractionates in plasma and serum with exosomes (Arroyo et al., 2011). We also provide evidence that miR-146a copurifies with exosome vesicles isolated from supernatant of Toxoplasma-infected cells (Figures S5C–S5E) or brain (data not shown), suggesting that the cell hosting the parasite might not be the only cell where miR-146a is operating as a microRNA.

**Cyst Burden Promotes miR-155 and miR-146a Induction in the Brains of Chronically Infected Mice**

Notwithstanding the ability of both type II and III strains of *T. gondii* to establish latent infections, our data strongly indicate that the induction of miR-146a or miR-155 was restricted to mice with high chronic cyst burdens (Figure 4). This potentially raises the question of whether ROP16 might be involved in this process, i.e., whether the kinase, by suppressing miR-146a induction (Figure 3) may contribute to lessen the cyst burden in the brain. We therefore monitored the course of chronic toxoplasmosis in mice infected with either ME49 or SF18 (ROP16-I) and SF28 (ROP16-II), two mouse-avirulent F1 progeny derived from the type II cross (Behnke et al., 2011) that were initially characterized as low and high inducers of miR-146a expression in stromal cells (Figure 2A). Our aim was to assess whether the ability of ROP16 to reduce miR-146a levels in the brain might impede or promote cystogenesis. We first confirmed that SF28 differs from SF18 by a strong induction of miR-146a in the brain (Figure 5A), thus mimicking the phenotype seen in stromal cells (Figure 2A) whereas miR-155 was equally induced by both strains.
and at the same level as the parental ME49 strain (Figure 5A). Moreover, mice infected with the SF18 strain (ROP16-I allele) had markedly reduced cyst burden in the brain, compared with mice infected with ME49 (type II control) or SF28 (ROP16-II allele) strains (Figure 5B). These results mirror those using infection of mice with a type III strain that was typified by a low number of cysts and no induction of miRNAs whatsoever in the brain (Figures 4C and 4E).

**miR-146a Ablation Promotes Host Resistance to Toxoplasma Infection in Susceptible C57BL/6 Mice**

miR-146a expression, while being efficiently induced by Toxoplasma, was shown to be persistently suppressed by strains expressing type I or III ROP16 alleles (Figures 4C and 5A). Given the established role of the ROP16 in generating innate resistance to oral challenge (Jensen et al., 2013), we decided to explore whether miR-146a influences host resistance during infection. As reported before, challenge of C57BL/6 mice with a transgenic type II strain expressing ROP16-I lead to better survival compared to a control infection with the parental type II Pru A7 strain (Figure 5C). To assess whether miR-146a was also required for host protection, we challenged miR-146a<sup>-/-</sup> knockout mice (described in Boldin et al., 2011) with 5 × 10<sup>5</sup> parasites of Pru A7, and their survival was compared with that of C57BL/6 control mice. miR-146a<sup>-/-</sup> mice infected with the Pru A7 WT strain were more resistant as evidenced by the 40% survival during the acute and chronic state of infection (50 days p.i., Figure 5C). However, this did not fully recapitulate the phenotype of the type II (Pru A7) strain expressing ROP16-I (80% survival of challenged mice), thereby indicating that additional ROP16-regulated genes might contribute to promote host resistance to Toxoplasma infection in susceptible C57BL/6 mice. Histopathological analysis of brains showed that miR-146a<sup>-/-</sup> mice challenged with Pru A7 developed a mild meningoencephalitis, whereas WT mice had a more severe toxoplasmic encephalitis (TE) characterized by the presence of significant numbers of parasites and areas of necrosis (data not shown).

Possibly, miR-146a<sup>-/-</sup> mice challenged with Pru A7 survived longer to TE than WT C57BL/6 mice because of changes in the inflammatory response to the parasite. Indeed, amounts of IFN-γ were significantly lower in sera and peritoneal exudates of infected miR-146a<sup>-/-</sup> mice compared to their WT littermates (Figure 5E). However, levels of other proinflammatory cytokines like tumor necrosis factor (TNF)-α, IL-12, IL-1β, and IL-6 did not change significantly (data not shown). In vitro assay using tail-derived fibroblasts confirmed the above in vivo experiment and showed that Toxoplasma (Pru A7) infection failed to induce IFN-γ gene transcription in miR-146a<sup>-/-</sup> tail-derived fibroblasts (Figure S5G). The early difference in IFN-γ production correlates well with high parasite burden in peritoneal exudates of WT animals (Figure 5F), which subsequently may affect parasite dissemination, load in the brain, and, ultimately, survival. It is noteworthy that infection by a type II strain expressing a ROP16-I allele led to a similar attenuation of IFN-γ mRNA expression to what observed in the absence of miR-146a (Figure S5G). Overall, these data indicate that miR-146a is required for full inflammatory response in mice during toxoplasmosis.

**DISCUSSION**

As obligate intracellular parasites, apicomplexans deploy sophisticated mechanisms to profoundly reorganize the host cell for their own needs. miRNAs were recently deemed as important components of gene regulatory networks that play critical roles in fine-tuning the host response to infection by several parasites of the phylum (Hakimi and Cannella, 2011). Here, we provide further evidence that the alteration of the microRNAome mediated by apicomplexan infection, both in terms of the repertoire and the magnitude, is different from one parasite to another. Remarkably, we uncovered a reservoir of host genetic riboregulators whose expression was tightly regulated by Toxoplasma infection. We thus showed that Toxoplasma infection increased the levels of miR-146a and miR-155 in the brains of mice infected by type II strains, which are competent to form cysts in the neural tissue. In this regard, miR-146a<sup>-/-</sup> ablation did not alter miR-155 levels in the brain of mice challenged with type II strain, suggesting that miR-155 induction in this tissue is miR-146a independent (Figure S5F). It is worth mentioning here that the levels of miR-146b were neither induced by Toxoplasma infection (Figure 1D) nor by the ablation of its paralog miR-146a (Figure S5F), which illustrates the uniqueness of this RNA signature that typifies type II parasite persistence in the host.

Both miR-155 and miR-146a are regulated by the transcription complex NF-κB, a component of a well-studied antipathogen pathway. The increase in the levels of miR-146a and miR-155 is not, however, a broad NF-κB-dependent inflammatory response to Toxoplasma infection. Several arguments support this assertion. First, the prominent upregulation of their activation was only evident at day 20 p.i. when the cysts were readily detectable and persisted in the chronic phase (Figures 4A and 4B). Second, other microRNAs whose expression was shown to be dependent on NF-κB pathway activation, e.g., miR-132 (Taganov et al., 2006) and miR-125b (Zhou et al., 2009), were not induced in the brain of chronically infected mice (Figure 4A). Finally, Plasmodium berghei ANKA strain, which causes cerebral malaria, selectively increases the levels of miR-150 but not those of miR-155 in the brain of CBA mice p.i. (El-Assaad et al., 2011), in quite the opposite fashion to infection by Toxoplasma (Figure 4A), suggesting that each apicomplexan infection is associated with a conserved, disease-specific signature of differentially expressed miRNAs.

We have clearly shown that the timing of miR-155 and miR-146a induction in the mice brains coincides with the presence of “mature” cysts and not with the mere occurrence of parasites (Figure 4B), suggesting that bradyzoite-specific effector(s) might operate in the host cell to regulate their levels. Meanwhile, we provided strong evidence that GRA15, while directly modulating the NF-κB pathway (Rosowski et al., 2011), did not alter the levels of miR-146a, nor of miR-155 (Figure S3). Another candidate dense granule protein, GRA24, which directly activates the MAPK p38α (Braun et al., 2013) pathway that interfaces with NF-κB, did not appear to be associated with the phenotype (data not shown).

Ablation of miR-146a expression in mice seems to play a role in the control of early proliferation of Toxoplasma in the gut, which was associated with a significant decrease in IFN-γ levels.
in sera and peritoneal contents postinfection (Figures 5E and 5F). These early inflammatory events may lead to lower CNS colonization and cyst burdens in miR-146a−/− mice, which result in a less severe encephalitis and delayed time to death as shown in Figure 5C. Therefore, the higher survival rate of miR-146a−/− mice might be more likely a consequence of early differences in parasite burden rather than a direct effect of miR-146a action in the brain.

Nevertheless, infection by type II strains, that are prone to cyst formation, systematically causes a significant increase in miR-146a levels in mice brain. However, our results do not reveal the primary mRNA target of the microRNA in the cyst-containing brain tissue. This could involve something akin to what has been reported in Treg cells, where miR-146a acts as a negative-feedback regulator to restrain IFN-γ-mediated pathogenic Th1 responses by targeting directly STAT1 (Lu et al., 2010). IFN-γ is the central cytokine in resistance against Toxoplasma during both the early and late stages of the infection. However, excessive or inappropriate activation of the IFN-γ pathway can be deleterious to the host. Thus, it appears that overproduction of IFN-γ in the genetically susceptible mice following Toxoplasma infection can lead to tissue destruction in small intestine and liver (Liesenfeld et al., 1999). Toxoplasma has developed means to prevent excessive host damage due to this cytokine. In this respect, SOCS-1 is induced during Toxoplasma infection and contributes to the inhibition of IFN-γ signaling by the parasite (Zimmermann et al., 2006). miR-146a may represent an alternative regulatory mechanism to fine-tune the IFN-γ-mediated inflammatory response throughout the chronic infection in mouse. In this model, miR-146a could directly target STAT1 protein translation or alternatively its upstream negative regulators like SOCS-1, the latter being previously identified as a downstream target of its coregulated counterpart, miR-155 (Cardoso et al., 2012).

In this study, we discovered a characteristic microRNA fingerprint typified by miR-146a and miR-155 that might be involved in the establishment of latent Toxoplasma infection through the buffering of the immune response in the brain of the infected host. Part of the variability of disease outcome in human infections may also be tied to this strain-specific microRNA signature. In North America and Europe, most human toxoplasmosis cases are mainly due to type II strains that exhibit a typical miR-146a/miR-155 signature in mice models. In contrast, type III strains that are more common in animals and rarely associated with human disease failed to induce these microRNAs in mice brain. Therefore, it will be important from a clinical point of view to establish if the aforementioned microRNA signature plays a role in determining severity of toxoplasmosis in humans.

**EXPERIMENTAL PROCEDURES**

**Mouse and Experimental Infection**

Six-week-old Swiss 01F and BALB/cJR or 9-week-old C57BL/6JR mice were obtained from Charles River Laboratories. Nine-week-old female B6(FVB)/mir146tm1.1Bal/J (http://jaxmice.jax.org/strain/016239.html) and their wild-type littersmates (http://jaxmice.jax.org/strain/000664.html) were obtained from The Jackson Laboratory. Mouse care and experimental procedures were performed under pathogen-free conditions in accordance with established institutional guidance and approved protocols from the Institutional Animal Care and Use Committee. The infection protocols are detailed in Supplemental Experimental Procedures.

**T. gondii Transfection**

Parasites were transfected using electroporation parameters established previously (Braun et al., 2013).

**Western Blot Analysis and Immunofluorescence Microscopy**

Immunofluorescence assays and western blots were performed as described (Braun et al., 2013) using antibodies listed in Supplemental Experimental Procedures.

**Cell Fractionation**

Cell fractionation was detailed in Supplemental Experimental Procedures.

**MicroRNA Microarray Analyses**

HFF cells were left uninfected or infected (MOI = 3) for 6–18 hr with RH or ME49 tachyzoites. RNA was isolated using TRIzol reagent (Invitrogen), followed by phenol:chloroform:isoamyl extraction. Total RNAs were profiled by Exiqon (http://www.exiqon.com) using miRCURY LNA arrays. Further processing of RNAs, hybridization, and analysis of microarray data are in Supplemental Experimental Procedures.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, five figures, and two tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2014.02.002.

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