Innate Resistance against *Toxoplasma gondii*: An Evolutionary Tale of Mice, Cats, and Men

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Recent studies have revealed remarkable species specificity of the Toll-like receptors (TLRs) TLR11 and TLR12 and the immunity-related GTPase (IRG) proteins that are essential elements for detection and immune control of *Toxoplasma gondii* in mice, but not in humans. The biological and evolutionary implications of these findings for the *T. gondii* host-pathogen relationship and for human disease are discussed.

Introduction

Host resistance and parasite virulence travel together through evolutionary time in a dynamic and often unstable equilibrium, each imposing unpredictable and intense selective pressures on the other. As a result, host-parasite relationships become remarkably specialized. General mechanisms of host immunity are now well understood, but for the parasite it is the details that matter, generating refined mechanisms of coadaptation specific for each host-parasite complex.

Toxoplasma gondii is a ubiquitous protozoan that belongs to the phylum Apicomplexa, and provides an interesting example of parasite-host coadaptation and successful transmission in nature (Elmore et al., 2010). The *T. gondii* sexual stages show restricted host specificity for feline species that act as definitive hosts. As a coccidian, what is unique in the *T. gondii* life cycle is the existence of an intermediate host. Felines are indiscriminate in their diets, and the asexual stage of *Toxoplasma* is notable for its lack of host specificity, infecting hundreds of avian and mammalian species and thus favoring parasite spread in nature and transmission to the definitive host. The evolutionary importance of any intermediate host for *Toxoplasma* is a function of the frequency with which it contributes to the transmission of the parasite or, in other words, is prey for cats. A reasonable thesis is that this role falls particularly (though certainly not exclusively) on the rodents that are thought to be natural intermediate hosts for this parasite. As a result the rodent immune system adapted to better cope with *T. gondii* infection. By contrast, many vertebrates that are not regularly part of the felines' food chain are considered accidental intermediate hosts. Humans, at least outside Kruger National Park, are certainly accidental intermediate hosts for *Toxoplasma* and play little or no part in its natural history and evolution. Thus the human immune system is not under selective pressure from *Toxoplasma*.

Toxoplasma sexual reproduction within feline gut epithelial cells generates oocysts that become highly infective when shed into the environment. After ingestion by the intermediate host, the parasite transforms into tachyzoites that rapidly multiply by endodyogeny within parasitophorous vacuoles (PVs) of a large number of different cell types. If not controlled by the immune system, the tachyzoites cause a systemic and lethal disease. The immunological control of tachyzoites is accompanied by the development of slowly replicating bradyzoites that persist isolated from the immune system in intracellular cysts typically residing in the central nervous system (CNS) and muscle. The life cycle is completed when an intermediate host infected with tissue cysts is eaten by a feline (Figure 1).

The more recent coevolution of *T. gondii* with rodents suggests that the murine immune response is well adapted to handle this parasite. Indeed, experimental infection of mice with *T. gondii* has become a widely exploited model for elucidating mechanisms of innate and acquired immunity to intracellular pathogens. However, since murine rodents, including the house mouse, are almost certainly evolutionarily significant hosts for *T. gondii* (at least in Eurasia, where they are native), they should be under selective pressure from the parasite, resulting in significant modification of the immune system. Indeed, recent findings show that humans and mice employ distinct innate immune pathways to control *T. gondii* infection. As reviewed here, elements required both for the initial detection and immune control of *T. gondii* in mice: the Toll-like receptors (TLRs) TLR11 and TLR12, and the immunity-related GTPase (IRG) proteins, respectively, are notably absent in humans. (There is a discrepancy in the designation of *TLR11* and *TLR12* gene/proteins. Here we follow the nomenclature defined in the functional studies of these TLRs [Yarovinsky et al., 2005; Andrade et al., 2013; Koblansky et al., 2013]. According to this definition, *TLR11* is a pseudogene, and *TLR12* is absent in the human genome. Both TLRs are functional in mice. In the NCBI GenBank definition, what we designate as TLR11 is defined as TLR12, and vice versa.) The alternative mechanisms that may replace these functions in humans, and the potential
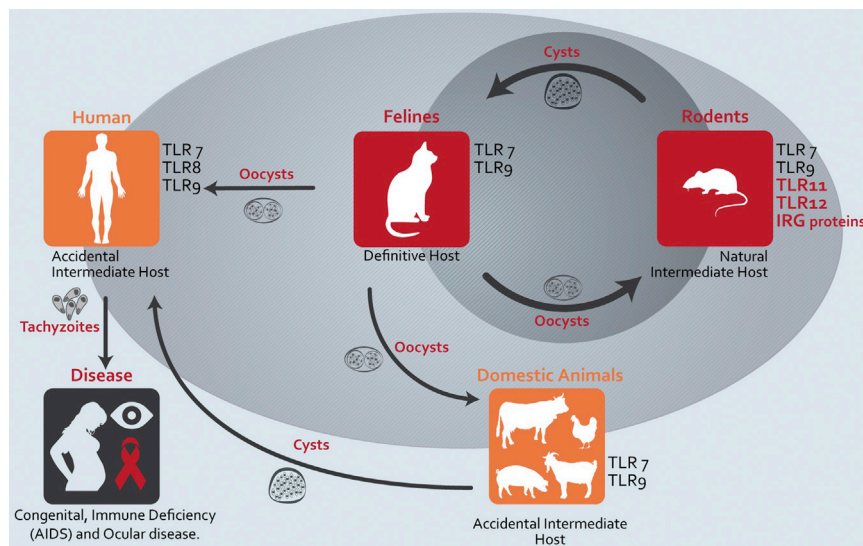
Figure 1. *Toxoplasma gondii* Life Cycle and the Host Specificity of TLR11/TLR12/IRG Proteins

Felines and rodents are the definitive and natural intermediate hosts for *T. gondii*, respectively. After sexual reproduction in intestinal epithelial cells, the oocysts are formed and shed to the environment in cat feces. Once in the environment the oocysts sporulate and become highly infective to a wide variety of vertebrates, including rodents and accidental intermediate hosts such as humans. The parasite life cycle is completed when the cat eats its prey, most often small rodents, and becomes infected by ingestion of the dormant *Toxoplasma* cysts. While most infections are asymptomatic, toxoplasma infection is a threat when congenitally transmitted to the fetus in immunologically naive pregnant women or in immunocompromised individuals. The wide distribution of TLR7 and TLR9 in different species, as well as host specificity of TLR11, TLR12, and diverse IRG system in rodents, is indicated.

consequences for human disease, are discussed along with the implications of these findings for the evolution of host defense pathways.

Mechanisms of Innate Immunity to *T. gondii* Infection in Mice

Mice deficient in essential common downstream elements of the TLR signaling pathway such as IRAK4 or MyD88 show impaired resistance to *T. gondii* due to a deficient cytokine response responsible for the control of the parasite (Béla et al., 2012; Scanga et al., 2002). TLR7 and TLR9 detect parasite RNA and DNA, whereas TLR11 and TLR12 sense the tachyzoite derived profilin-like protein (PRF) (Andrade et al., 2013; Koblansky et al., 2013; Yarovinsky et al., 2005). TLR-activated dendritic cells (DCs) (Andrade et al., 2013; Goldszmid et al., 2012) and inflammatory monocytes (MØs) (Dunay et al., 2008) produce IL-12 that triggers the production of IFN-γ by NK cells and later by T lymphocytes. IFN-γ also primes innate immune cells to express proinflammatory mediators. This inflammatory milieu favors the development of Th1 lymphocytes that are the basis of the long-term host control of *T. gondii* (Gazzinelli and Denkers, 2006).

Two main IFN-γ-inducible mechanisms by which mouse cells limit tachyzoite growth have been studied. One of these is mediated by the inducible nitric oxide synthase (NOS2) and the release of nitric oxide (NO) by MØs (Hunter and Sibley, 2012). The second mechanism, which is essential for mice survival upon infection with *T. gondii*, involves the IRG proteins that are inducible by IFNγ both in nonmyeloid and myeloid cells. The recruitment of members of this family of large GTPases results in rupture of the PV membrane and tachyzoite degradation in the host cell cytoplasm (Howard et al., 2011).

Based on recent studies, we hypothesize that TLR11 and TLR12 emerged in rodents as *T. gondii* sensors that more efficiently initiate IL-12-dependent IFNγ production, leading to a rapid activation of IRG proteins (Figure 2). Thus, while structurally distinct, these two systems are connected to each other and serve a common purpose, which is the control of the rapidly

multiplying tachyzoites at the very first days of infection. While TLR11 and TLR12 sense a conserved *Toxoplasma* protein, it is the IRG proteins that deal with parasite diversity. As a consequence, the *TLR11* and *TLR12* genes are conserved, whereas IRG proteins interact antagonistically with polymorphic virulence factors of *Toxoplasma* and are themselves highly polymorphic.

TLR11/TLR12 Specificity and Function

TLR11 was shown to mediate mouse resistance to Gram-negative bacteria that are relevant to human disease (Mathur et al., 2012). Nevertheless, considering the central role of rodents in the *T. gondii* life cycle and that TLR11 responds to minute amounts (in the range of 1–10 ng/ml) of PRF (Yarovinsky et al., 2005), we favor the hypothesis that this TLR emerged as a consequence of the selective pressure of *T. gondii* or perhaps closely related coccidian parasites. Experiments performed with genetically manipulated parasites lacking PRF indicate that this protein is involved in tachyzoite motility and host cell invasion (Plattner et al., 2008). Hence, TLR11 senses a protein that is essential for parasite survival and perpetuation in the host and, thus, highly conserved among different strains of *Toxoplasma.*

Two recent studies also indicate that TLR12, the TLR most closely related to TLR11, is also required for innate recognition of *Toxoplasma* tachyzoites (Andrade et al., 2013; Koblansky et al., 2013). Both TLR11 and TLR12 are expressed by DCs, and the lack of either dramatically reduces IL-12 production in response to PRF in vitro and results in impaired IL-12 production in *T. gondii*-infected mice. Moreover, upon activation with PRF, TLR11 and TLR12 form heterodimers that may represent the dominant form of the two proteins involved in signaling in myeloid DCs (Andrade et al., 2013). Intriguingly, both TLR11 and TLR12 are necessary for the activation of macrophages and CD11c+ DCs by PRF, whereas TLR12 alone is sufficient for activation and type I IFN production by Flt3+ plasmacytoid DCs exposed to PRF (Koblansky et al., 2013).

Another important observation is that the response to PRF is completely ablated in DCs from mice functionally mutated in
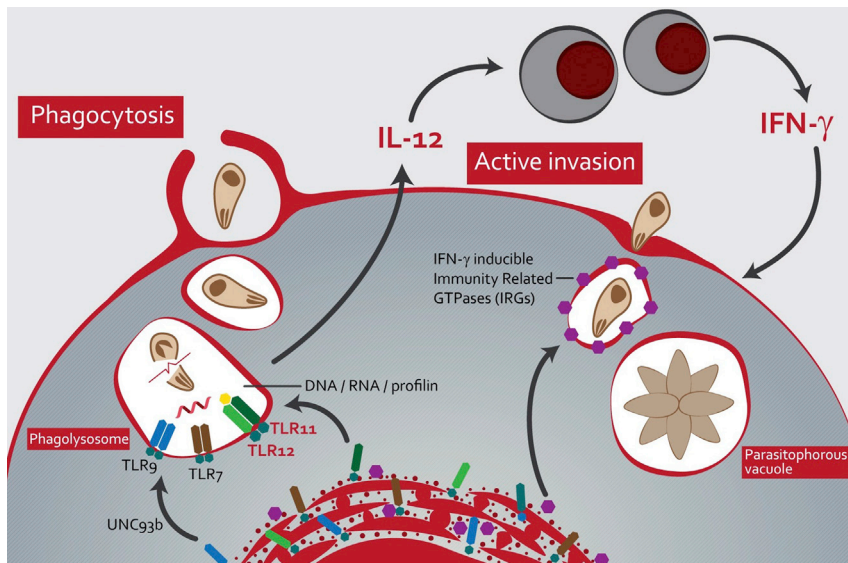
Figure 2. The TLR11, TLR12, and IRG Protein Loop Is Essential for Mouse Resistance to _Toxoplasma_ Infection
Uptake of parasite debris or, alternatively, destruction of engulfed tachyzoites leads to activation of TLR7, TLR9, and TLR11/TLR12 heterodimers by _Toxoplasma_ RNA, DNA, and profilin (PRF) released in phagolysosomes. The activation of these innate immune receptors, and in particular TLR11 and TLR12, induces the production of IL-12 by dendritic cells and consequent induction of IFNγ by NK cells and T lymphocytes. IFNγ then leads to expression of IRG proteins that are recruited to the parasitophorous vacuole and destroy _T. gondii_ tachyzoites that have actively invaded the host cells.

UNC93B1 which encodes for a chaperone required to bring nucleic acid sensing TLRs 3, 7, and 9 from the endoplasmic reticulum (ER) to the endocytic system. These animals are highly susceptible to _Toxoplasma_ infection (Melo et al., 2010). Nevertheless, it was demonstrated that TLR11 and TLR12 also colocalize with UNC93B1, both in the ER and in the endosomal compartment, suggesting that the extreme phenotype of the _UNC93B1_ mutant mice is due to a combined defect in the nucleic acid-sensing and PRF-sensing TLRs. Consistent with this hypothesis, triple KO mice deficient in TLRs 7, 9, and 11 closely resemble _UNC93B1_ mutant mice in their complete loss of resistance to _T. gondii_ (Andrade et al., 2013).

IRG Proteins: Specificity and Function
IRG proteins are a family of IFNγ-inducible mouse GTPases with an approximate molecular weight of 47 kDa that are essential for the control of tachyzoite replication and host survival in mice infected with _T. gondii_ (Howard et al., 2011). While the _IRG_ gene family is widely distributed in vertebrates, the _IRG_ gene diversity found in rodents is remarkable. Mouse IRG proteins fall into five subfamilies, IRGA, IRGB, IRGC, IRGD, and IRGM, encoded by about 23 genes in the C57BL/6 genome. All except _IRGC_ are massively inducible in all nucleated cells by IFNγ. Disruption of individual _IRG_ genes results in a profound (_Irgm1_, _Irgm3_) or partial (_Irgd_, _Irga6_) enhancement of susceptibility to _T. gondii._ The three members of the IRGM (GMS) subfamily, Irgm1, Irgm2, and Irgm3, are negative regulators of the IRGA, IRGB, and IRGD effector (GKS) subfamilies that damage the PV membrane. The regulatory GMS proteins prevent off-target activation on organellar membranes (Haldar et al., 2013). Members of the effector GKS subfamilies assemble on the PV membrane. This becomes corrugated, limiting the internal volume available for the parasite and pulling it tight against the elastic cytoskeleton of the parasite. The PV membrane ultimately ruptures, releasing the tachyzoite into the cytosol (Zhao et al.,

2009), where it dies within 20 min. The infected cell itself undergoes necrotic death about an hour later. For unknown reasons, some PVs are not coated with IRG proteins and become permissive to parasite replication. The survivors are probably destined to differentiate into bradyzoites and encyst in brain and muscle tissues.

The IRG system can also defend itself against attack by _T. gondii_ virulence factors. Certain strains of _T. gondii_ secrete a pseudokinase (ROP5) and a kinase (ROP18) forming a complex that phosphorylates and inactivates IRG proteins, resulting in high virulence (Hunter and Sibley, 2012). Some mouse strains are resistant to the ROP5/ROP18 kinase complex of these virulent _T. gondii_ strains, which they achieve via an IFNγ-inducible "tandem" IRG protein of the IRGB subfamily built of two GTPase units, which serve as a decoy for the ROP18/ROP5 kinase complex, sparing the effector IRG proteins (Lilue et al., 2013). Indeed, the IRG system of the mouse is at least as polymorphic as the MHC, indicating the presence of selection pressures acting on this system. The _IRG_ allele-specific differential resistance of mouse strains to certain _T. gondii_ strains supports the argument that _T. gondii_ may be responsible for at least some of this pressure on the IRG system.

While IRG proteins of the mouse appear to play no role in resistance to many bacterial and protozoal pathogens, _T. gondii_ is not the only organism that can impact on the IRG system and potentially exert selection pressure. Resistance of mice to certain strains of chlamydia also requires an intact IRG system (Howard et al., 2011; Miyairi et al., 2007). It is therefore to be expected that the IRG system will provide immunity against other groups of organisms.

Lack of Functional _TLR11_, _TLR12_, and _IRG_ Genes in the Human Genome
While we have learned a lot about how mice control _T. gondii_ infections, how do humans respond to this parasite? Although normally asymptomatic in healthy individuals, _T. gondii_ infection is a serious and often fatal disease in immunodeficient individuals. In nonimmune pregnant women, congenital transmission may cause serious damage to the child before and after birth. In addition, in South America, eye disease and other symptoms of systemic inflammation are not uncommon outcomes of primary

infection, even in immunocompetent individuals. From the dramatically enhanced susceptibility of AIDS patients and patients on immunodepletion therapy it is clear that the adaptive immune system plays an essential role in immunity to human toxoplasmosis.

Less well understood is the nature or relevance of innate immunity against *T. gondii* infection in humans, a situation compounded by the absence in humans of the critical mediators of innate immunity discovered in the mouse. The segment of mouse chromosome 4 that contains the *TLR11* gene is found in human chromosome 1, but the human *TLR11* gene contains three stop codons and does not encode a functional protein. Similarly, the *TLR12* gene, present in mouse chromosome 14, is not present in the human genome. Thus, recognition of PRF, which is a strong activator in the mouse, apparently plays no role in human resistance to infection. Nevertheless, it is possible that the nucleic acid-sensing TLRs play an important role in *Toxoplasma* recognition by human cells. Consistent with this hypothesis, human monocytes and DCs are activated by parasite ssRNA and DNA, probably via TLR7, TLR8, and TLR9, to produce large amounts of proinflammatory cytokines (Andrade et al., 2013).

More puzzling, however, is the absence from humans of the IRG system, the essential effector mechanism against *T. gondii* in mice. The IFNγ-inducible IRG proteins are encoded in two gene clusters on mouse chromosomes 11 and 18. These clusters are in genomic segments syntenic with a region of human chromosome 5. The only vestige of the entire IRG family in this region is a fragment carrying most of a G domain with typical sequence features of the IRGM regulatory subfamily of the mouse. Humans and mice share one orthologous and highly conserved full-length *IRG* gene, *IRGC*, expressed exclusively in the testis in both species, which is not regulated by cytokines and is unlikely to play a role in host resistance to *T. gondii* (Bekpen et al., 2005).

Human cells appear to dispose of a range of IFNγ-inducible toxoplasmastatic mechanisms, but it remains unclear which, if any, are essential for the usually benign course of *T. gondii* infection. Both NO-dependent and NO-independent control mechanisms (Nagineni et al., 1996) have been reported in different systems. There is also considerable evidence for a link between the interferon-inducible tryptophan catalyzing enzyme, indoleamine dioxygenase (IDO), tryptophan depletion, and a toxoplasmastatic activity in a variety of human cellular systems (Pfefferkorn, 1984). However, these activities are not universally observed in human cells, suggesting that an unidentified NO- and IDO-independent mechanism induced by IFNγ may also play an important role in human resistance to *T. gondii* infection.

Although absent in humans, one can imagine that TLR11, TLR12, and IRG proteins in other animals may indirectly influence human disease by controlling the zoonotic dissemination of the parasite. These elements operating together in small rodents should select and strongly impact the abundance of different parasites strains in the environment, and thereby the ones that most often infect humans. However, a major gap in our present information and parasite transmission to the intermediate hosts is the nature of resistance against *T. gondii* in the domestic cat and other felines, which expresses neither TLRs 11 and 12 nor IRG proteins.

Evolution and Host Specificity of TLR11, TLR12, and IRG Proteins

Considering the ubiquity of *Toxoplasma* in nature, it is intriguing that genes encoding TLR11 and TLR12, as well as IRG proteins, are not found in many mammalian species. Interestingly, if the representation of *IRG* gene numbers is superimposed on the species that encode *TLR11* and *TLR12* genes, some consistency is apparent, particularly with the rodents (Figure 3A). When first infected with *T. gondii*, felines shed around 100 million oocysts that may persist in the environment for years. In addition, parasite genetic crosses in intestinal epithelial cells from cats often generate virulent clones (Saeij et al., 2007). Due to intimate contact with felines, small rodents, the natural intermediate hosts, are often exposed to higher doses of and more virulent parasites than other groups of mammals. It may therefore be that TLR11, TLR12, and the polymorphism of IRG proteins have been positively selected for in rodents and other small mammals, because of their critical importance in host resistance against high infective doses or more virulent clones of *T. gondii*.

The *TLR*s comprise an ancient multigene family that fits better with a "Swiss army knife" model of evolution. Each TLR subfamily evolved to detect components of a distinct nature that are essential for viability of different categories of microorganisms and therefore cannot easily mutate. Because of their critical importance for host resistance to microbial pathogens, TLRs are kept under selective pressure to maintain their specificity, and thus selection is dominant over mutation (Roach et al., 2005). While these genes are evolving slowly, the evolutionary changes seen in the TLR repertoire may reflect changes in the spectrum of species-specific pathogens and structural adaptations to relevant microbial components, as is the case for PRF recognition by TLR11 and TLR12 in rodents.

It is most likely that *TLR11* diverged from the *TLRs 21-23* genes found in fish and frogs. Interestingly, *TLR11* and *TLR12* are the most divergent of all *TLR* genes and are found only in mammals, suggesting that they are relatively new and fast-evolving genes (Roach et al., 2005). The platypus genome contains an open reading frame with 40% homology to mouse *TLR11*, which is likely the mammalian ancestral gene for *TLR11*. A limited number of mammal species encode functional *TLR11* and *TLR12* genes, which include rodents (mice, rats, degu, and hamsters) and other orders of small mammals (Lagomorpha, Erinaceomorpha, and Soricomorpha) that are potential prey for cats and other felines. The high similarity and remarkable overlapping distribution in different species favor the hypothesis that *TLR12* is a duplication of *TLR11* and the two genes coevolved to exert a similar function. Consistently, the three species that have *TLR11* pseudogenes lack the *TLR12* gene (Figure 3). Published studies indicate that TLR11 and TLR12 work primarily as a heterodimer (Andrade et al., 2013). Therefore, it is possible that in the absence of TLR12, the TLR11 gene was lost because it could not function as a homodimer.

The *IRG* gene family is more widely distributed in vertebrates, is present in fish and mammals, but apparently is absent in birds. In mammals, *IRG* genes appear in all orders, but erratically; for example, they are represented in the dog genome and absent from the cat. The *IRG*s are, however, consistently well represented and in large gene numbers in rodents. Since control of the mouse IRG system requires all three members of the IRGM
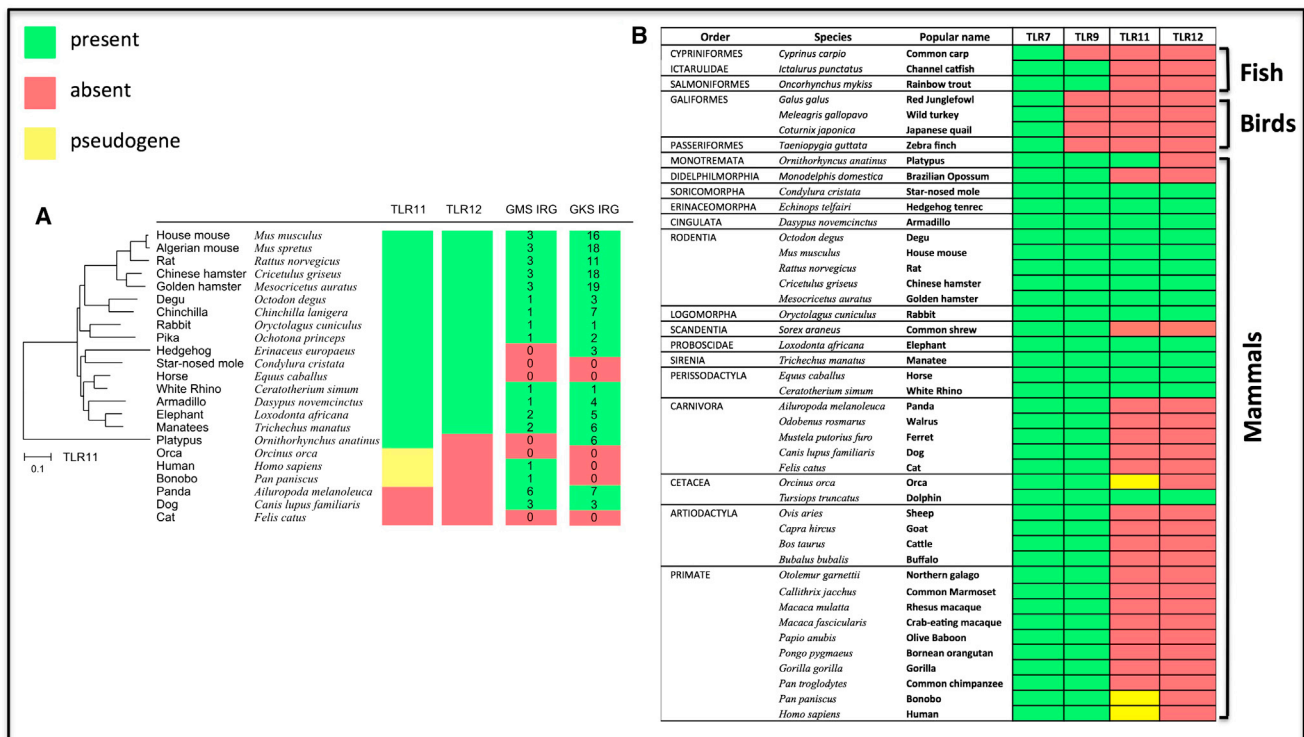
Figure 3. Distribution of *TLR11*, *TLR12*, and *IRG* Protein Genes in Mammalian Species
(A) Phylogenetic tree of *TLR11* and overlapping expression of *TLR12* as well as *IRG GMS* and *IRG GKS* gene families.
(B) Ubiquitous versus confined distribution of *TLR7/TLR9* and *TLR11/TLR12* genes in vertebrate genomes, respectively. TLR7, TLR9, TLR11, and TLR12 coding sequences were downloaded from GenBank from species that have complete genome sequences. Retrieved sequences from mammals, fishes, and birds were analyzed by ORFfinder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html) to identify pseudogenes by looking for frameshifts or premature stop codons. Multiple and global sequence alignments for each *TLR* were performed by Muscle. Neighbor-joining trees were designed by MEGA 5.2.2 from muscle alignments.

regulator subfamily, and since the system is weakened by loss of individual IRG effector proteins, we cannot interpret a genome such as that of American pika or the rabbit, which appear to contain only one *IRGM* gene and only one or two effector *IRG* genes, and we cannot say whether these reduced sets can function in immunity against *T. gondii*. Where the IRG system is absent or nonfunctional, as in humans, we assume that other mechanisms effectively control the parasite.

Because *Toxoplasma* PRF is a conserved molecule, TLR11/TLR12 heterodimers are likely to sense most genetic variants of *T. gondii* that continuously attack rodents. In contrast, IRG diversity seems necessary to protect mice against the activity of the highly polymorphic members of parasite ROP proteins. Consistently, certain IRG haplotypes confer resistance to *T. gondii* strains described as highly virulent in laboratory mice (Lilue et al., 2013), generating a host phenotype that allows encystment and transmission of otherwise lethal clones. These data suggest that the generation of diversity in the *T. gondii* ROP proteins is a continuous selective force for IRG protein evolution in the wild, even if the selective balance in this complex system is unclear. The interacting surfaces of the IRG and ROP5 proteins are the most polymorphic regions of both proteins (Fleckenstein et al., 2012; Lilue et al., 2013), also consistent with the argument that these proteins exert reciprocal selection pressures on each other. Because diverse rodent species have

many *IRG* genes (Figure 3A), we speculate that the arms race with ROP proteins started in an ancestral species and is still a dynamic process in contemporary rodents. One could predict that the arms race would be dampened in geographic areas where few clonal populations of *Toxoplasma* became dominant. However, the presence of *TLR11* and *TLR12* and a sizeable collection of *IRG* genes in the elephant and other larger species unlikely to be evolutionarily significant for *T. gondii* seem to argue against such an oversimplified view, and one cannot discard a more complex scenario in which a constellation of infections together imposes a special demand on expansion of IRG families in small rodents.

Although one can imagine the evolutionary pressures that gave rise to TLR11, TLR12, and the diversification of the IRG system, a major question remains unanswered (Lauw et al., 2005). Why are these genes absent or downgraded to noncoding status in the genome of humans and other vertebrates? A likely explanation is that only recently, after cat domestication, did *T. gondii* become a relevant pathogen for humans and other domestic animals. As a consequence, these hosts did not coevolve with *Toxoplasma*, and thus have not been under selective pressure to develop these highly specific mechanisms of host defense to develop against the parasite (Figure 1).

An alternative explanation for the absence of TLR11, TLR12, and IRG host defense mechanisms in a range of vertebrates

would be the deliberate deletion of the relevant genes. Certainly *IRG* genes, which are already present in fish, were removed from the primate lineage sometime before the evolution of monkeys. While found only in mammals, it is clear that *TLR11* gene has downgraded to a pseudogene in several species. In addition, both *TLR11* and *TLR12* genes are absent in the genomes of more recently evolved mammals (Figure 3).

Perhaps a deleterious inflammatory response that follows activation of TLR11 and TLR12 carries a net cost in the absence of intense infection, while activation of the IRG system can induce host cell death by necrosis and could also be detrimental in certain species. Figures 1 and 3B illustrate the wide distribution in vertebrates of TLR7 and TLR9 that recognize both *Toxoplasma* RNA and DNA. Hence, it is possible that the nucleic acid-sensing TLRs are sufficient to control parasite replication in the initial stages of infection in many mammal species, including perhaps felines that are critical to the *Toxoplasma* life cycle. As we have seen, the IRG system is not the only effector system available that mediates control of *T. gondii* infection. Hence, negative selection of *TLR11*, *TLR12*, and *IRG* genes/functions may contribute to host tolerance to infection and represent a gain of net fitness cost, unless intense exposure to *T. gondii* and other relevant infections impose unusual pressure demanding the maintenance of these unique immunological functions for host survival.

Regardless, the studies reviewed are consistent with a model in which the coevolution of *T. gondii* and rodents led to the emergence of *TLR11*, *TLR12*, and a family of diversified *IRG* genes as highly specialized innate immune mechanisms of host resistance to this parasite. Further studies on rodent toxoplasmosis in which powerful parasite genetic and host immunologic tools can be readily deployed should help provide new insights into this fascinating example of parasite-host coadaptation.

REFERENCES

Andrade, W.A., Souza, Mdo.C., Ramos-Martinez, E., Nagpal, K., Dutra, M.S., Melo, M.B., Bartholomeu, D.C., Ghosh, S., Golenbock, D.T., and Gazzinelli, R.T. (2013). Combined action of nucleic acid-sensing Toll-like receptors and TLR11/TLR12 heterodimers imparts resistance to *Toxoplasma gondii* in mice. Cell Host Microbe *13*, 42–53.

Bekpen, C., Hunn, J.P., Rohde, C., Parvanova, I., Guethlein, L., Dunn, D.M., Glowalla, E., Leptin, M., and Howard, J.C. (2005). The interferon-inducible p47 (IRG) GTPases in vertebrates: loss of the cell autonomous resistance mechanism in the human lineage. Genome Biol. *6*, R92.

Béla, S.R., Dutra, M.S., Mui, E., Montpetit, A., Oliveira, F.S., Oliveira, S.C., Arantes, R.M., Antonelli, L.R., McLeod, R., and Gazzinelli, R.T. (2012). Impaired innate immunity in mice deficient in interleukin-1 receptor-associated kinase 4 leads to defective type 1 T cell responses, B cell expansion, and enhanced susceptibility to infection with *Toxoplasma gondii*. Infect. Immun. *80*, 4298–4308.

Dunay, I.R., Damatta, R.A., Fux, B., Presti, R., Greco, S., Colonna, M., and Sibley, L.D. (2008). Gr1(+) inflammatory monocytes are required for mucosal resistance to the pathogen *Toxoplasma gondii*. Immunity *29*, 306–317.

Elmore, S.A., Jones, J.L., Conrad, P.A., Patton, S., Lindsay, D.S., and Dubey, J.P. (2010). *Toxoplasma gondii*: epidemiology, feline clinical aspects, and prevention. Trends Parasitol. *26*, 190–196.

Fleckenstein, M.C., Reese, M.L., Könen-Waisman, S., Boothroyd, J.C., Howard, J.C., and Steinfeldt, T. (2012). A *Toxoplasma gondii* pseudokinase inhibits host IRG resistance proteins. PLoS Biol. *10*, e1001358.

Gazzinelli, R.T., and Denkers, E.Y. (2006). Protozoan encounters with Toll-like receptor signalling pathways: implications for host parasitism. Nat. Rev. Immunol. *6*, 895–906.

Goldszmid, R.S., Caspar, P., Rivollier, A., White, S., Dzutsev, A., Hieny, S., Kelsall, B., Trinchieri, G., and Sher, A. (2012). NK cell-derived interferon-γ orchestrates cellular dynamics and the differentiation of monocytes into dendritic cells at the site of infection. Immunity *36*, 1047–1059.

Haldar, A.K., Saka, H.A., Piro, A.S., Dunn, J.D., Henry, S.C., Taylor, G.A., Frickel, E.M., Valdivia, R.H., and Coers, J. (2013). IRG and GBP host resistance factors target aberrant, "non-self" vacuoles characterized by the missing of "self" IRGM proteins. PLoS Pathog. *9*, e1003414.

Howard, J.C., Hunn, J.P., and Steinfeldt, T. (2011). The IRG protein-based resistance mechanism in mice and its relation to virulence in *Toxoplasma gondii*. Curr. Opin. Microbiol. *14*, 414–421.

Hunter, C.A., and Sibley, L.D. (2012). Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. Nat. Rev. Microbiol. *10*, 766–778.

Koblansky, A.A., Jankovic, D., Oh, H., Hieny, S., Sungnak, W., Mathur, R., Hayden, M.S., Akira, S., Sher, A., and Ghosh, S. (2013). Recognition of profilin by Toll-like receptor 12 is critical for host resistance to *Toxoplasma gondii*. Immunity *38*, 119–130.

Lauw, F.N., Caffrey, D.R., and Golenbock, D.T. (2005). Of mice and man: TLR11 (finally) finds profilin. Trends Immunol. *26*, 509–511.

Lilue, J., Müller, U.B., Steinfeldt, T., and Howard, J.C. (2013). Reciprocal virulence and resistance polymorphism in the relationship between *Toxoplasma gondii* and the house mouse. Elife *2*, e01298.

Mathur, R., Oh, H., Zhang, D., Park, S.G., Seo, J., Koblansky, A., Hayden, M.S., and Ghosh, S. (2012). A mouse model of *Salmonella typhi* infection. Cell *151*, 590–602.

Melo, M.B., Kasperkovitz, P., Cerny, A., Könen-Waisman, S., Kurt-Jones, E.A., Lien, E., Beutler, B., Howard, J.C., Golenbock, D.T., and Gazzinelli, R.T. (2010). UNC93B1 mediates host resistance to infection with *Toxoplasma gondii*. PLoS Pathog. *6*, e1001071.

Miyairi, I., Tatireddigari, V.R., Mahdi, O.S., Rose, L.A., Belland, R.J., Lu, L., Williams, R.W., and Byrne, G.I. (2007). The p47 GTPases Iigp2 and Irgb10 regulate innate immunity and inflammation to murine *Chlamydia psittaci* infection. J. Immunol. *179*, 1814–1824.

Nagineni, C.N., Pardhasaradhi, K., Martins, M.C., Detrick, B., and Hooks, J.J. (1996). Mechanisms of interferon-induced inhibition of *Toxoplasma gondii* replication in human retinal pigment epithelial cells. Infect. Immun. *64*, 4188–4196.

Pfefferkorn, E.R. (1984). Interferon gamma blocks the growth of *Toxoplasma gondii* in human fibroblasts by inducing the host cells to degrade tryptophan. Proc. Natl. Acad. Sci. USA *81*, 908–912.

Plattner, F., Yarovinsky, F., Romero, S., Didry, D., Carlier, M.F., Sher, A., and Soldati-Favre, D. (2008). Toxoplasma profilin is essential for host cell invasion and TLR11-dependent induction of an interleukin-12 response. Cell Host Microbe *3*, 77–87.

Roach, J.C., Glusman, G., Rowen, L., Kaur, A., Purcell, M.K., Smith, K.D., Hood, L.E., and Aderem, A. (2005). The evolution of vertebrate Toll-like receptors. Proc. Natl. Acad. Sci. USA *102*, 9577–9582.

Saeij, J.P., Coller, S., Boyle, J.P., Jerome, M.E., White, M.W., and Boothroyd, J.C. (2007). Toxoplasma co-opts host gene expression by injection of a polymorphic kinase homologue. Nature *445*, 324–327.

Scanga, C.A., Aliberti, J., Jankovic, D., Tilloy, F., Bennouna, S., Denkers, E.Y., Medzhitov, R., and Sher, A. (2002). Cutting edge: MyD88 is required for resistance to *Toxoplasma gondii* infection and regulates parasite-induced IL-12 production by dendritic cells. J. Immunol. *168*, 5997–6001.

Yarovinsky, F., Zhang, D., Andersen, J.F., Bannenberg, G.L., Serhan, C.N., Hayden, M.S., Hieny, S., Sutterwala, F.S., Flavell, R.A., Ghosh, S., and Sher, A. (2005). TLR11 activation of dendritic cells by a protozoan profilin-like protein. Science *308*, 1626–1629.

Zhao, Y.O., Khaminets, A., Hunn, J.P., and Howard, J.C. (2009). Disruption of the *Toxoplasma gondii* parasitophorous vacuole by IFNgamma-inducible immunity-related GTPases (IRG proteins) triggers necrotic cell death. PLoS Pathog. *5*, e1000288.