The impact of *Toxoplasma gondii* on the mammalian genome

Urs B Müller¹ and Jonathan C Howard¹,²,³

Nobody doubts that infections have imposed specialisations on the mammalian genome. However sufficient information is usually missing to attribute a specific genomic modification to pressure from a specific pathogen. Recent studies on mechanisms of mammalian resistance against the ubiquitous protozoan parasite, *Toxoplasma gondii*, have shown that the small rodents presumed to be largely responsible for transmission of the parasite to its definitive host, the domestic cat, possess distinctive recognition proteins, and interferon-inducible effector proteins (IRG proteins) that limit the potential virulence of the parasite. The phylogenetic association of the recognition proteins, TLR11 and TLR12, with *T. gondii* resistance is weak, but there is evidence for reciprocal polymorphism between parasite virulence proteins and host IRG proteins that strongly suggests current or recent coevolution.

Addresses

¹Institute for Genetics, University of Cologne, Zülpicher Strasse 47a, 50674 Cologne, Germany
²Instituto Gulbenkian de Ciência, Rua da Quinta Grande 6, 2780-156 Oeiras, Portugal
³Max Planck Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, 50829 Cologne, Germany

Corresponding author: Howard, Jonathan C (j.howard@uni-koeln.de)

**Infection modifies genomes**

The mammalian genome has clearly been influenced by infection. The extraordinary genomic complexity of the rearranging receptors of lymphocytes and the complex array of immune functions assembled in the mammalian MHC are testimony to millions of years of pathogen pressure.

Less straightforward is to document where and how specific pathogens have triggered specific genomic effects. Recent fatal pandemics have left their marks on the human genome, for example in the shape of a number of more or less dysgenic alleles of α-globin and β-globin for malaria, witnessing the urgency and intensity of selection by novel pathogens. In mice the superantigenic ORF proteins of endogenous mammary tumor viruses appear to have taken a toll of T cell receptor VB families [1] as the selective priorities for the mouse seem to have favoured a sub-optimal T cell repertoire over the risk of inflammatory death.

The strongest evidence for a definite recent causal relationship between specific features of pathogen and host genomes is reciprocal polymorphism with an experimentally demonstrable causal chain. Apart from the classic examples noted above, this level of analysis has only occasionally been achieved, and most notably in plant disease resistance [2**]. When experimental data has been substantiated by ecological evidence, one may fairly describe such a scenario as co-evolution.

**The evolutionarily significant host (ESH)**

The pattern of host–pathogen co-evolution depends on the extent to which host resistance reduces pathogen transmission. Fast-evolving pathogens counter this cost by rapid evasive evolution. These familiar ‘Red Queen’-like processes can result in polymorphic variation in host and pathogen as each attempts to sidestep the other. *Toxoplasma gondii* (*T. gondii*) is an extremely promiscuous pathogen, generating recombinational diversity through gametogenesis in all species of true cats, and all warm-blooded animals are potentially intermediate hosts (see Figure 1). Evolutionary significance of hosts for *T. gondii* is therefore not yes or no, but a quantitative parameter. Some species are certainly in this sense important hosts for transmission of the pathogen, others probably not, a distinction applying equally to definitive and intermediate hosts. In a comparison between important and less important hosts we might identify genomic signatures of the immune resistance machinery that reflect selective pressure from the parasite on the ESH.

The evolution of *T. gondii* will be driven in the foreseeable future by its relationship with the domestic cat as definitive host, but the absolute dominance of the domestic cat is recent and it is unknown whether any genomic coadaptation has already occurred. The limited genotypic diversity of *T. gondii* in the Old World compared with S. America may reflect an ancient S. American origin for the species [3] although there are arguments against this view [4]. In any case, the original genetic diversity of Old World *T. gondii* may have been
larger and the recent expansion of the domestic cat, an Old World species until the sixteenth century, may have favoured a specific subset of pre-adapted genotypes.

The identification of dominant ESH species as intermediate hosts is more complex, but mammal or bird species that are rare or inaccessible as prey for domestic cats must be low down on the hierarchy, while species that are abundant and accessible are high up. Humans, on the other hand, while abundant and globally infected by *T. gondii* at a rate over 1% per year of age [5*], are inaccessible as prey for domestic cats and can be eliminated as an ESH. The parasite is completely uninterested in defeating, or being defeated by, human immunity. In the event, while human immunity is normally sufficient to reduce morbidity from *T. gondii* infection to very low levels, the parasite’s exceptional ability to use host immunity in general as a trigger for bradyzoite conversion means that infected humans do carry cysts and so far no immunity sufficient for parasite elimination has yet been recorded in man. What we may fairly say is that no components of human immunity seem to be specifically dedicated to resistance against *T. gondii*. The human genome thus seems to provide a reasonably reliable negative control.

What about the strong ESH candidates? Cat and mouse are global species and sympatric. Furthermore, foraging mice should have a significant chance of ingesting oocysts spread in cat feces. Infection rates in urban *Mus musculus* above 50% have been reported in the UK [6], but much lower rates (0–5%) are more general [7–10]. In US studies, infection of wild *M. musculus* is reported to be in the low range (0–3% [11,12]) and values for the US native mouse, *Peromyscus*, are similar [13]. Since unconfined domestic cats defecate and hunt in the natural environment adjacent to their homes, rather than at home, the ecology of *Apodemus* and other local wild-life may be more relevant to the evolution of modern *T. gondii* in Europe than that of *M. musculus*. Significant infection rates have been reported in the European field mouse, *Apodemus* [14,15] as well as in voles and shrews [8,16], abundant Eurasian small mammals often found near human habitation but scarcely overlapping in range with the domestic mouse. Likewise, domestic cats regrettably catch the

The *Toxoplasma gondii* life cycle, highlighting the distinction between evolutionarily relevant hosts and irrelevant hosts. The infected domestic cat excretes oocysts into the environment with the feces where they are inadvertently ingested by foraging animals. Humans may be infected via contact with cat feces in the domestic environment and in other ways. After a brief proliferative phase (tachyzoite) immunity may develop and restricts further growth. In this case the parasite undergoes a phase change to a slowly-replicating form (bradyzoite), building cysts in brain and muscle that persist for the life of the intermediate host. If the intermediate host is in the food-chain of domestic cats, as common birds and small mammals are, then it is relevant for transmission and of evolutionary relevance to the parasite. Infected hosts outside the food chain of the domestic cat, like humans, are irrelevant for transmission. The parasite must stimulate an effective immunity shortly after infection to ensure avirulence; failure to do so allows uncontrolled proliferation and the early death of the host. Virulence is clearly costly for the host and in mice and probably other small rodents. The highly polymorphic IRG system of IFNγ-inducible GTPases provides resistance against *T. gondii* strains of difference virulence without achieving sterile immunity. Presumably the selective advantage of the high virulence for mice, seen in some strains of *T. gondii*, reflects pressure on the parasite from other evolutionarily relevant hosts that can indeed achieve sterile immunity during primary infection. So far, high virulence has always been attributed to the ROP5/ROP18 complex of virulence proteins, known to target IRG proteins (see Figure 2), so it is likely IRG proteins are also required for the induction of sterile immunity in such hosts.
common wild songbirds that live with us, as well as unloved but abundant urban feral rock pigeons. These may also be important ESH species, but it is certain that a significant proportion of T. gondii pass through M. musculus during the generational cycle and the mouse is certainly the best-known candidate for a species with significant ESH credentials.

**Immune mechanisms against Toxoplasma in mice and humans**

Two striking differences between mouse and man have been highlighted, a recognition mechanism and an effector mechanism. In mice, innate recognition of T. gondii infection depends on two members of the TLR (Toll-like receptor) family, TLR11 and TLR12, probably forming a heterodimer [17], and the trigger was identified as T. gondii-profilin [18]. Both TLR proteins are absent in human [18,19]. Without them, mice are susceptible to normally avirulent T. gondii strains. Secondly, members of a family of 10–20 interferon-γ-inducible GTPases, the IRG proteins [20], assemble on and disrupt the parasitophorous vacuole membrane [21]. IRG proteins are essential for mouse survival from normally avirulent T. gondii infection [22,23]. Humans express only one non-inducible IRG fragment, IRGM, of uncertain function. A further family of interferon-inducible GTPases, the 65 kDa guanylate binding proteins (GBP), is present in both species. In the mouse GBPs assemble on a proportion of IRG-loaded parasitophorous vacuoles [24] and contribute to the strength of IFNγ-inducible resistance [25–27]. In the human, GBPs do not assemble on parasitophorous vacuoles although a resistance function distant from the vacuole has been proposed [28*].

Much of the immune machinery involved in resistance against T. gondii is, however, common to man and mouse, forming the general innate-adaptive response axis: macrophages, dendritic cells, IL-12, IFNγ, CD4 and CD8 T cells, CD40, the MHC, as well as NO and active oxygen radicals are all implicated in resistance against T. gondii [29,30]. It was shown recently that polyubiquitin is deposited on the vacuolar membrane in both mouse and human cells [31*,32*,33*]. Human resistance against T. gondii is remarkably effective despite the absence of TLR11/12 and IRG proteins. Tryptophan depletion by the catabolic action of an IFNγ-inducible indoleamine dioxygenase has been implicated in restricting T. gondii growth in human cells [34], but this has not been generalizable over cell types and culture conditions [35]. The human NLRP1 inflammasome has also been implicated as an initiator of some cell-autonomous immunity in human macrophages in the absence of IFNγ [36], but the effector mechanism is unknown. Human TLR5 has recently been shown to be triggered by T. gondii-profilin [37], arguably replacing TLR11/12. Perhaps immunity of humans against T. gondii is the sum of small effects. Certainly the human mechanism in its entirety does not exist in mice since loss of IRG or TLR11/12 proteins is fatal. Thus we have a clear dichotomy: mice have the essential TLR11/12 and IRG mechanisms but not the human mechanisms, while humans have their mechanisms, whatever they may be, but not the TLR11/12 and IRG mechanism.

**What do these differences mean?**

The known specializations of the mouse accompany its ESH status [38,39*]. It was further recently found that IL-12 production in mice is triggered by live parasite invasion, in human by phagocytosis [40] and the authors state: ‘possibly reflecting a direct involvement of rodents and not humans in the parasite life cycle.’ However while mouse is an ESH and human not, no causal connection has been offered. Gazzinelli and colleagues [39] tried to strengthen the link, both for TLR11/12 and for IRG proteins, by looking at a wider range of species, but no convincing correlation emerged. IRG genes were certainly most abundant in small rodents, but nearly absent in rabbits. Horses, an unlikely prey for small cats, have no recorded IRG genes but their relative abundance in elephants and manatees stretches the correlative argument too thin. The same problem afflicts the TLR11/12 distribution, present in rodents and lagomorphs, but also in horses, rhinos, elephants and manatees. Absent or pseudogenised in humans, orcas, dogs and cats, the expression of TLR11/12 seems to associate inversely with carnivory, but this correlation too is destroyed by their absence also from the obligate herbivore, the giant panda.

**Reciprocal polymorphism strengthens the argument**

The correlative argument contains no causal link. If, however, host and pathogen show reciprocal polymorphism in virulence and resistance, it would suggest that the system is under selection. In the TLR11/12 recognition/response mechanism against T. gondii-profilin, no relevant polymorphism was found correlating with infection status in Apodemus [41*]. However in the IRG system in mice, there is functional reciprocal polymorphism with T. gondii virulence proteins [42]. Eurasian T. gondii strains designated type I are virulent for laboratory mice, for example, C57BL/6. Differential virulence of T. gondii strains is due to allelic variation in two homologous, polymorphic secreted proteins, a kinase (ROP18) and a pseudokinase (ROP5). Together, these phosphorylate two conserved threonines on effector IRG proteins and inactivate them [43]. Type I strains are, however, resisted by a wild-derived mouse strain, CIM, from South India [42]. In crosses between CIM and C57BL/6, all the resistance maps to a highly polymorphic IRG gene cluster on chromosome 11. The polymorphic surface of the pseudokinase ROP5 binds the nucleotide-binding domain of Irg6 adjacent to the target threonines [42,44]. This surface region, on the homologous Irgb2-b1 protein encoded on chromosome 11, is the same region that shows evidence...
of recent directional selection. Irgb2-b1 from the CIM mouse transfected into C57BL/6 cells blocks phosphorylation of Irga6 by a virulent type I strain of T. gondii.

In this analysis, the argument favouring a causal chain from virulence to resistance is complete. The polymorphic variation of Irgb2-b1 ‘matches’ the polymorphic variation of parasite ROP5 and the results have biological meaning. Type I strains that are virulent in mice carrying the laboratory mouse allele of Irgb2-b1 kill their host within a few days and thereby essentially eliminate the chance of their own transmission. In mice carrying the CIM allele of Irgb2-b1, however, both parasite and host profit; the parasite can encyst in a resistant host, while the host lives out a normal life (Figure 2).

These results leave us with a number of questions. It has recently been shown that much of the virulence of S. American T. gondii strains for laboratory mice is also due to alleles expressed at ROP5 [45**]. Can we conclude that the allelic variation in ROP5 across multiple parasite strains globally is all directed at allelic variants of IRG proteins? Or are different ROP5 alleles directed at entirely different target proteins relevant to different ESH species? The house mouse is a Eurasian species, yet most of the polymorphic variation in ROP5 is found among the enormous diversity of S. American strains [35]. Which species are ESHs in S. America and what if any IRG proteins do they have? Since the resistant allele of Irgb2-b1 is advantageous to both host and parasite at least in Eurasia, why is it not fixed? What selection pressure has led to the evolution of the susceptible Irgb2-b1 allele of the laboratory mouse strains and to its greatly reduced expression level (unpublished results Lilue & Muller?}

Just as the polymorphic virulence factors of T. gondii may have different molecular targets in different ESH species, so the IRG resistance system is certainly not directed
The impact of Toxoplasma gondii on the mammalian genome Müller and Howard 23

exclusively at T. gondii. Polymorphic variants of the IRG system found among laboratory mouse strains also regulate resistance to Chlamydia trachomatis [46] and Chlamydia psittaci [47], while IRG proteins are also essential for resistance of mouse cells against the microsporidian fungus, Encephalitozoon cuniculi, although differential resistance has not been shown for IRG alleles [48]. Both Chlamydyales and Microsporidia are ubiquitous and abundant pathogen classes and may well be more important for the evolutionary dynamics of the IRG system than Toxoplasma.

The struggle for avirulence

The strategy of T. gondii as a parasite is based on a quest for avirulence, a capacity to attenuate but not to destroy the immune resistance of the host, thus securing the permanent residence required to await transmission. How the parasite achieves this ideal state in thousands of potential hosts, with strikingly different immune systems is the major unknown in T. gondii biology. This power is analogous to the ability of the adaptive immune system of vertebrates to resist thousands of different pathogens. The adaptive immune system shows little co-adaptation at a genomic level to different pathogens; it is a general anti-pathogen machine. Likewise, T. gondii has a general anti-host machine, not perfect, but able to titrate host immunities of many different kinds against the self-destructive potential of its own replicative powers. Armed with this instrument, whatever it consists of, is presumably irrelevant whether a specific host species is an ESH or not. Polymorphic variation in ROP5 and ROP18 is essential in mice, that use the IRG system, but irrelevant in humans, that do not [35]. Presumably the polymorphism and regulation of other genetic systems are essential against different immune resistance mechanisms favoured by other host species. T. gondii sometimes fails to achieve its goal of avirulence in geographically incoherent infections; some strains of S. American T. gondii are highly virulent in humans [49], a non-native species, and many S. American strains are highly virulent for laboratory mice, which represent W. European M. m. domesticus >90% genetically [50]. Likewise, the type I Eurasian strains relatively frequent in the far East are highly virulent for laboratory mouse strains but avirulent for M. m. castaneus strains from the East Asian region. These instances hint at further co-evolution between T. gondii and its intermediate hosts.

Conclusion

For the moment the proven relationship between polymorphic virulence alleles of T. gondii and the proven resistance alleles of the IRG system of mice presents the strongest evidence that this host pathogen-pair are now or have recently been in a dynamic co-evolutionary relationship of sufficient intensity to contribute to genome modification through allelic diversification by both partners. The weak correlation in species distribution of the TLR11/12 pair possibly suggests that this recognition system also helps or has recently helped several mammals in immunity against T. gondii, but does not tell us that selection by this organism has brought it into existence, any more than that it is likely that H-2L d, for example, which is known to present several T. gondii peptides to T cells [51,52] owes its existence to the parasite, and there is no evidence yet in either case of a dynamic co-evolutionary process at work.

T. gondii has evolved a complex orchestra of actions that play on vertebrate pathogen resistance machinery and except in the case of the IRG system there is little reason to believe that host resistance machinery is anything other than beneficial to both host and parasite in enabling the avirulent state and encystment. The polymorphism of Irgb2-b1 and its intimate association with the virulence polymorphism of ROP5 and ROP18 raises the question, what selection generates type I virulence in T. gondii strains where M. musculus is an ESH? Avirulent types II and III strains can also encyst in the highly resistant CIM mice so there is no ‘need’ for extra virulence. Arguably, the type I virulent strains are preferentially adapted to another important ESH species, perhaps a rat, whose IRG system is capable of enforcing sterile immunity on strains lacking the virulent alleles of ROP5 and ROP18. The Irgb2-b1 allele of mice would then be accounted for as an essential adaptation for mice living within the range of such virulent strains, perhaps typically in the Far East. Polymorphic variation in the IRG system is probably also driven by other parasites as well, for example Chlamydia or Microsporidia. Allelic frequencies will depend on the ratio of the intensity of selection pressures from the parasites.

Much work will be required and at many different analytical levels, genetic, biochemical, structural, ecological and immunological, to clarify these issues.

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