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Phil. Trans. R. Soc. Lond. B 1997 **352**, 1355-1359
doi: 10.1098/rstb.1997.0120

Mechanisms of innate resistance to *Toxoplasma gondii* infection

J. ALEXANDER[1], T. M. SCHARTON-KERSTEN[2], G. YAP[2], C. W. ROBERTS[1], F. Y. LIEW[3] AND A. SHER[2]

[1]*Department of Immunology, University of Strathclyde, 31 Taylor Street, Glasgow G4 0NR, UK*
[2]*Immunobiology Section, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA*
[3]*Department of Immunology, University of Glasgow, Glasgow G11 6NT, UK*

SUMMARY

The interaction of protozoan parasites with innate host defences is critical in determining the character of the subsequent infection. The initial steps in the encounter of *Toxoplasma gondii* with the vertebrate immune system provide a striking example of this important aspect of the host–parasite relationship. In immunocompetent individuals this intracellular protozoan produces an asymptomatic chronic infection as part of its strategy for transmission. Nevertheless, *T. gondii* is inherently a highly virulent pathogen. The rapid induction by the parasite of a potent cell-mediated immune response that both limits its growth and drives conversion to a dormant cyst stage explains this apparent paradox. Studies with gene-deficient mice have demonstrated the interleukin-12 (IL-12)-dependent production of interferon γ (IFN-γ) to be of paramount importance in controlling early parasite growth. However, this seems to be independent of nitric oxide production as mice deficient in inducible nitric oxide synthase (iNOS) and tumour necrosis factor receptor were able to control early growth of *T. gondii*, although they later succumbed to infection. Nitric oxide does, however, seem to be important in controlling persistent infection; treating chronic infection with iNOS metabolic inhibitors results in disease reactivation. Preliminary evidence implicates neutrophils in effector pathways against this parasite distinct from that described for macrophages. Once initiated, IL-12-dependent IFN-γ production in synergy with other proinflammatory cytokines can positively feed back on itself to induce 'cytokine shock'. Regulatory cytokines, particularly IL-10, are essential to down-regulate inflammation and limit host pathology.

1. INTRODUCTION

Toxoplasmosis is of clinical and veterinary importance, being not only a major cause of congenital disease and abortion in man and his domestic animals but also a life-threatening opportunistic infection in immunodepressed individuals such as transplant recipients and AIDS patients, where it is the single major cause of cerebral mass lesions (Luft & Remington 1992; Ambroise-Thomas & Pelloux 1993). In immunocompetent individuals, however, infection normally results in a mild to asymptomatic infection, as a potent innate T-cell-independent immune response is generated against the rapidly dividing stage of the life cycle, the tachyzoite (Sher *et al.* 1993; Gazzinelli *et al.* 1993, 1994; Hunter *et al.* 1993). This response results not only in tachyzoite killing but also in the parasite's transforming to the dormant encysted bradyzoite stage within skeletal or heart muscle and the central nervous system. Generally, immunity to infection is lifelong and parasite latency is maintained by an adaptive type 1 T-cell response (reviewed by Alexander & Hunter 1997).

2. INNATE CONTROL OF PARASITE GROWTH

Early studies with neutralizing antibodies or recombinant interferon γ (rIFN-γ) clearly demonstrated the importance of IFN-γ during both the acute and chronic stages of infection (Gazzinelli *et al.* 1992; McCabe *et al.* 1984; Suzuki *et al.* 1988, 1989, 1990). The critical importance of this cytokine can be seen in the total inability of mice deficient in IFN-γ and IFN-γ receptor (IFN-γR) to survive the early stages of acute infection (Scharton-Kersten *et al.* 1996; Alexander *et al.* 1996) (table 1). Studies of mice with severe combined immunodeficiency (SCID) demonstrated the source of IFN-γ to be natural killer (NK) cells (Sher *et al.* 1993; Gazzinelli *et al.* 1993; Johnson 1992; Hunter *et al.* 1993; Schluter *et al.* 1993). The production of IFN-γ by NK cells is dependent on interleukin 12 (IL-12) (Gazzinelli *et al.* 1993) and consequently the survival of *T. gondii*-infected SCID mice is enhanced after administration of this cytokine, whereas treatment with anti-IL-12 (Gazzinelli *et al.* 1993) results in earlier mortality

Table 1. *Comparison of the percentage survival plus brain cyst burden between cytokine-deficient or cytokine-receptor-deficient mice and their wild-type counterparts*

(All infections were with 20 tissue cysts of *T. gondii*. Cyst burdens in the brain were measured day 21 (†) or day 28 (*) after infection. n.d., not done. Adapted from Alexander *et al.* (1996), Alexander & Hunter (1997), Gazzinelli *et al.* (1996), Roberts *et al.* (1996) and Scharton-Kersten *et al.* (1996, 1997).)

			survival (%)					
			day 12		day 20		cyst burden (±s.e.m.)	
cytokine/receptor deficient	*T. gondii*	mouse	+/+	−/−	+/+	−/−	+/+	−/−
IL-4	RRA	B6/129	70	25	70	25	16264 ±2998	7244* ±1071
IL-6	RRA	129SVJ	100	100	85	30	1355 ±59	10450* ±3350
IFN-γR	RRA	129SVeV	85	0	85	0	n.d.	n.d.
TNFαR1	RRA	129SVJ	85	85	85	0	n.d.	n.d.
IL-10	ME49	C57BL/10	100	25	100	0	n.d.	n.d.
IL-12	ME49	C57BL/6	100	0	100	0	n.d.	n.d.
IFN-γ	ME49	C57BL/6	100	10	100	0	n.d.	n.d.
iNOS	ME49	B6/129	100	100	100	65	2400 ±250	6000† ±1000

(Hunter *et al.* 1994; Gazzinelli *et al.* 1994). Furthermore, IL-12 p40 gene-deficient mice are as susceptible to *T. gondii* infection as those deficient in IFN-γ or its receptor (Scharton-Kersten *et al.* 1997) (table 1). It is, however, well known that non-activated macrophages are unable to produce IL-12 (Flesch *et al.* 1995; Sher *et al.* 1993; Gazzinelli *et al.* 1993). Nevertheless, mice can produce IL-12 during *T. gondii* infection in the absence of endogeneous IFN-γ (Scharton-Kersten *et al.* 1996). Therefore, during *T. gondii* infection IL-12 (from an unprimed cell source, for example dendritic cells) probably initiates NK-cell IFN-γ production, which in its turn induces macrophage IL-12 production, which further enhances the production of IFN-γ by NK cells. This production of IFN-γ is further amplified by the IFN-γ-induced production of macrophage tumour necrosis factor α (TNF-α) and probably IL-1 (Sher *et al.* 1993; Hunter *et al.* 1995*a*) in a positive feedback loop. The proinflammatory cytokines comprising the innate response collectively also influence the generation of the protective type 1 adaptive response (reviewed by Alexander & Hunter 1997).

3. CONTROL OF INFLAMMATION

The activation of NK cells that occurs early in infection is rapidly down-regulated (Goyal *et al.* 1988; Hauser *et al.* 1982) by the activity of regulatory cytokines such as IL-10 and transforming growth factor β (TGF-β). TGF-β directly inhibits IFN-γ production by NK cells (Hunter *et al.* 1995*b*; Bellone *et al.* 1995), whereas IL-10 inhibits NK cell activity indirectly by inhibiting monokine production by macrophages (D'Andrea *et al.* 1993; Rennick *et al.* 1992; Fiorentino *et al.* 1991). Thus, treating infected SCID mice with neutralizing antibodies against these cytokines increases the production of IFN-γ and prolongs survival (Sher *et al.* 1993; Hunter *et al.* 1995*b*). However, proinflammatory mediators such as IL-12, IFN-γ and TNF-α, which are essential in controlling parasite growth, can be detrimental to the host if produced in excess (reviewed by Clark *et al.* 1991) and their effects need to be counterbalanced by the simultaneous induction of regulatory cytokines. Thus, mice deficient in IL-10 (table 1) die during the acute stage of infection owing to the overproduction of IL-12 and subsequent CD4+ T-cell production of high levels of IFN-γ (Ellis Neyer *et al.* 1997; Gazzinelli *et al.* 1996). Similarly, mice deficient in IL-4, which downregulates IFN-γ production and activity, have a significantly increased mortality (table 1) (Roberts *et al.* 1996; Suzuki *et al.* 1996). In addition to IL-10, nitric oxide (NO) (Candolfi *et al.* 1994) has been identified as having an important regulatory role in limiting cachexia and decreasing tissue damage and death early during infection with *T. gondii*. IL-10 and NO do this by inhibiting the production of IL-2 and IFN-γ and by inducing lymphocyte hyporesponsiveness 7 d after infection (Candolfi *et al.* 1994; Khan *et al.* 1995).

4. ROLE OF REACTIVE NITROGEN INTERMEDIATES (RNIs)

From studies *in vitro*, IFN-γ is thought to mediate its antiparasitic effects in the murine model primarily by upregulating the expression of macrophage and microglial inducible nitric oxide synthase (iNOS) (Gazzinelli *et al.* 1993; Jun *et al.* 1993) and the production of NO (Langermans *et al.* 1992). NO is believed not only to be directly toxoplasmacidal (Langermans *et al.* 1992) but also to promote tachyzoite to bradyzoite transformation by inhibition of mitochondrial respiration (Bohne *et al.* 1993). Surprisingly, therefore, whereas mice treated with the reactive nitrogen intermediate inhibitor aminoguanidine succumbed to chronic infection they

Table 2. *Mean survival of* T. gondii *-infected mice, deficient in IFN-γ, IL-12 or iNOS, infected with 20* T. gondii *cysts*

(Adapted from Sharton-Kersten *et al.* (1997).)

knockout	treatment	mean survival (days)	maximum survival (days)
IFN-γ	—	10	10
IL-12	—	10	14
iNOS[a]	control sera	22	24
iNOS[a]	anti-IFN-γ	10	15
iNOS[a]	anti-IL-12	11	13

[a] iNOS knockout mice were injected intraperitoneally with 1 mg of normal rat immunoglobulin G, anti-IFN-γ monoclonal antibody X MG-6 or anti-IL-12 mAb C17.8 1 d before infection.

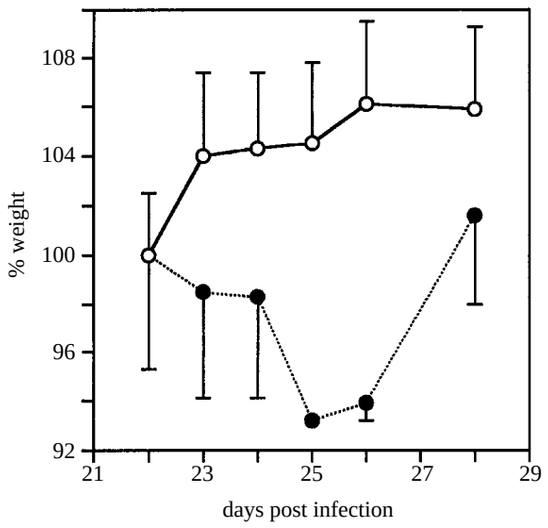

Figure 1. Disease severity as measured by weight loss in BALB/K mice infected with 20 cysts of *T. gondii* orally. Mice were otherwise untreated (○) or inoculated intraperitoneally with 250 mg kg^{-1} L-NMMA (●) on days 22–25 after infection.

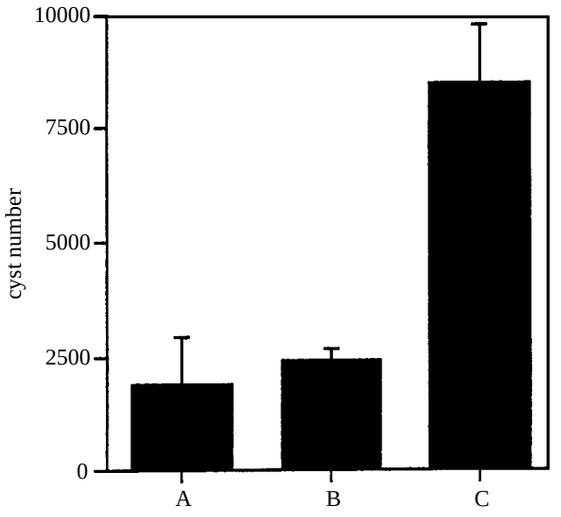

Figure 2. The brain cyst burden in BALB/K mice at day 30 after infection with 20 *T. gondii* cysts orally. Mice were either untreated (bar A) or treated intraperitoneally with 250 mg kg^{-1} L-NMMA at 6 h (bar B) or on day 22 (bar C) after infection.

survived acute infection (Hayashi *et al.* 1996; Khan *et al.* 1996).

One possible explanation is that tachyzoites are able to infect all nucleated cells thus bypassing effector mechanisms operating at the level of the macrophage *in vivo*. Furthermore, mice deficient in iNOS or the receptors for TNFα, which is an important cofactor for macrophage and microglial cell activation and NO production (Langermans *et al.* 1992; Chao *et al.* 1993; Jun *et al.* 1993), survive significantly longer than animals deficient in IFN-γ, IFN-γR or IL-12 (table 1) (Scharton-Kersten *et al.* 1997; Alexander *et al.* 1996). During the early period of infection these mice are able to control tachyzoite growth systemically, although macrophages from iNOS-deficient mice are totally deficient in their capacity to control parasite replication *in vitro* (Scharton-Kersten *et al.* 1997). Treating iNOS-deficient mice before infection with anti-IL-12 or anti-IFN-γ-neutralizing antibodies shortens their lifespan to the equivalent of IL-12−/− or IFN-γ−/− mice (table 2), demonstrating that innate immunity in both iNOS-deficient and wild-type mice is dependent on IL-12 and IFN-γ (Scharton-Kersten *et al.* 1997).

Current evidence suggests that RNIs have a more important role in controlling persistent infection (Scharton-Kersten *et al.* 1997; Hayashi *et al.* 1996) and treating chronically infected with the iNOS inhibitor L-NMMA (N^G-mononomethyl-L-arginine) results in the reactivation of infection and increased parasite burden (figures 1 and 2). Similar treatment at the onset of infection does not increase mortality nor does it result in increased severity of infection (results not shown) or cyst burdens (figure 2). Significantly, we have also shown that treatment of chronically infected mice with RNI inhibitors increases the severity of ocular toxoplasmosis (F. Roberts, C. W. Roberts and R. McLeod, unpublished work).

5. OTHER POSSIBLE EFFECTOR MECHANISMS CONTROLLING PARASITE GROWTH

Studies *in vitro* have also identified reactive oxygen intermediates as having toxoplasmacidal activity (Murray & Cohn 1979; Murray *et al.* 1979). However, p47 *phox*-deficient mice, which lack an inducible oxidative burst (Jackson *et al.* 1995), are able to control both the acute and chronic stages of *T. gondii* infection, and their macrophages can limit parasite growth *in vitro* (T. M. Scharton-Kersten, S. H. Jackson & S. M. Holland, unpublished work), arguing against a role for these radicals in parasite killing. Alternatively, IFN-γ has been shown to limit *T. gondii* replication in human fibroblasts (Pfefferkorn *et al.* 1986), macrophages (Murray *et al.* 1989) and glioblastoma cells (Daubener *et al.* 1996) by inducing the enzyme indolamine oxygenase, thus

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

starving the parasite of tryptophan. Nevertheless, this effector mechanism cannot be identified in mice (Schwartzman *et al.* 1990). Similarly, human platelets, via thromboxane production, have been shown to lyse parasitized cells (Yong *et al.* 1991), although again a similar mechanism has not been identified in mice.

However, depleting both wild-type mice and iNOS-deficient mice of granulocytes, which have also been shown to have toxoplasmacidal activity *in vitro* (Wilson & Remington 1979), by using the RB6-8C5 antibody, results in significantly more rapid death in both the intraperitoneal (Scharton-Kersten *et al.* 1997) and per-oral routes of infection (P. Sayles, personal communication). However, no differences in susceptibility were noted after eosinophil depletion by neutralization of IL-5, clearly implicating an effector role for neutrophils (Scharton-Kersten *et al.* 1997). Neutrophils have also recently been identified as having critical roles in the innate immune response against *Listeria monocytogenes* (Conlan & North 1991; Rogers & Unanue 1993) and *Candida albicans* (Romani *et al.* 1996) in mice. Significantly, therefore, we have found that IL-6-deficient mice, which have an impaired neutrophil response (Romani *et al.* 1996), are also more susceptible during acute infection than their wild-type counterparts (table 1). These NO-independent toxoplasmicidal mechanisms, which seem to operate through neutrophils, await characterization.

6. CONCLUSIONS

The recent availability of gene-deficient, as well as mutant, mice has allowed the detailed dissection of immunological pathways controlling infectious disease. In particular these studies have highlighted the importance of the innate immune response not only in controlling initial microbial growth but in directing the nature of the subsequent adaptive response. The intracellular protozoan parasite *T. gondii* is a particularly potent inducer of innate immunity, rapidly initiating the sequential potentiation of the inflammatory cytokine cascade. Studies with mutant and gene-knockout mice in particular have helped to identify the cells involved in this response, their products and how (and in what order) they interact to promote inflammation. However, the control of *T. gondii* infection during acute disease tends to be a delicate balance between the production of proinflammatory cytokines to control parasite growth and the concurrent production of regulatory cytokines to limit host pathology. Thus mice deficient in regulatory cytokines are as likely to succumb to acute infection as those lacking inflammatory cytokines. Studies with 'knockout mice' have also identified the relative importance of the products mediating protection and also when and where they might operate. Thus it has been recognized (Scharton-Kersten *et al.* 1997) that the control of acute *T. gondii* infection is dependent on IL-12 and IFN-γ, and independent of RNIs, although NO does have a role in controlling persistent infection. This has led ultimately to evidence implicating the participation of neutrophils in innate resistance to this protozoan that might involve effector pathways quite distinct from those postulated in the original paradigm of macrophage-mediated control of initial infection.

We thank the following for providing knockout mice: Dr Jeanne Magram (Hoffman-La Roche, Nutley, NJ, USA), Dr Michel Aguet (Genentech Inc, Palo Alto, CA, USA), Dr Horst Bluethmann (Hoffman-La Roche, Basel, Switzerland), Dr John MacMicking, Dr Carl Nathan (Cornell University Medical College, New York, USA) and Dr John Mudgett (Merck Research Laboratories, Rahway, NJ, USA).

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