EFFECT OF ROUTE OF INFECTION ON OUTCOME OF *TOXOPLASMA GONDII* INFECTION IN hu-PBL SCID MICE[#]

Desiree J. Meyer¹, Jane E. Allan¹ and Miles H. Beaman^{2,3,*}

¹School of Medicine and Pharmacology, University of Western Australia, Perth, Australia

²School of Biomedical, Biomolecular and Chemical Sciences, University of Western Australia, Perth, Australia

³Department of Medicine, University of Notre Dame Australia, Fremantle Campus, Western Australia, Fremantle, Australia

Received: January 9, 2013; Accepted: January 13, 2013

Toxoplasma gondii (*T. gondii*) causes serious infection, especially in immunocompromised hosts. The relevance of animal models of toxoplasmosis to human disease is unclear, but have indicated that the route of *Toxoplasma* infection may affect the outcome. A humanized model of toxoplasmosis of immunocompromised mice (i.e. hu-PBL SCID), using the intraperitoneal (IP) route demonstrated long-term engraftment of human cells and worsening of inflammation compared to controls. In this study, we examined the effect of route of infection on this hu-PBL SCID model using a *Toxoplasma* strain (i.e. DAG) isolated from an immunocompromised human. Oral infection led to an asymptomatic infection, whereas animals infected by the IP route succumbed more quickly to infection. Human cells, detected through species-specific β -actin mRNA, were not as prominent in IP-infected animals as compared to orally infected and uninfected animals. There was evidence of control of toxoplasmosis in some orally infected animals, and this was associated with the presence of human cells in multiple tissues. Thus, the route of infection dramatically affects the outcome of infection, either by affecting parasite replication or expansion of human immune cells. Further studies of oral *Toxoplasma* infection using hu-PBL SCID mice may help in developing chemotherapies and immunotherapeutic strategies for toxoplasmosis.

Keywords: route, infection, *Toxoplasma gondii*, hu-PBL SCID mice

Abbreviations: IP, intraperitoneal; hu-PBL, human peripheral blood lymphocytes; p.c., peritoneal cavity; PO, per oram; p.i., post-infection

Introduction

Toxoplasma gondii (*T. gondii*) infection is found worldwide and usually occurs after ingestion of tissue cysts in meat or oocysts in feline feces. Some human cases result from congenital (transplacental) infection or transfer in infected organ transplants. After ingestion, the cysts rupture and the emerging bradyzoites proliferate and become tachyzoites. Tachyzoites then encyst in host tissues (such as brain, muscle) and persist for the life of the host [1]. AIDS patients in particular are at risk of reactivation of cysts leading to severe toxoplasmosis with potentially fatal encephalitis. Infection of other immunocompromised in-

dividuals, including the fetus during pregnancy, also may result in fatalities or serious sequelae. Treatment relies on chemotherapeutic agents which can have deleterious effects on the patient with long-term use and are rarely active against the cyst stage of the organism (which is the source of reactivation).

Most animal models of toxoplasmosis use immunocompetent animals, predominantly mice, and *Toxoplasma* strains isolated from non-human hosts which were inoculated via non-physiological routes (intraperitoneal [IP], intracerebral). These models have identified the importance of macrophage and lymphocyte activating cytokines (including IFN- γ , TNF- α , IL-2, IL-7, IL-12, IL-15, and

*Corresponding author current address: Dr. M. H. Beaman, Director of Pathology, Western Diagnostic Pathology, 74 McCoy St. Myaree, Western Australia 6154, Australia; Phone: 61-8-9317-0999; Fax: 61-8-9317-1572; Mobile: 61-41-990-3457; E-mail: miles.beaman@wdp.com.au

[#]These data were presented in part at:

Immunoparasitology Symposium, Woodshole, USA, 1999.

British Society for Parasitology, Annual Meeting, Coventry, England, 1999.

Informed consent was obtained from patients. Experimentation followed guidelines of the Human Rights Committee, University of Western Australia.

IL-17) and T-lymphocytes in host defence against *T. gondii* (reviewed in [2]). The route of *T. gondii* infection (IP vs peroral [PO]) has been shown to affect the outcome of murine toxoplasmosis in differing inbred strains [3]. PO infection using a low virulence *T. gondii* strain (Me49) has been shown to have differential effects in mice, depending on which inbred strain was used, with C57BL/6 being particularly susceptible [4]. Mortality in C57BL/6 mice after PO infection appears to be due to severe necrosis of the small intestine which was shown to be CD4⁺ T cell-dependent and IFN- γ mediated [5]. Nevertheless, the relevance of these models to toxoplasma disease of immunocompromised human is not clear.

We have previously described a murine model based on the use of immunodeficient SCID mice into which human PBL are engrafted (hu-PBL SCID) 1 week prior to IP infection with the relatively avirulent DAG strain (which was originally isolated from an immunocompromised human) of *T. gondii* [6]. The grafts contain a mixture of monocytes, B and T lymphocytes which have the potential for interacting when challenged by specific antigens. Long-term engraftment of human B and T lymphocytes was documented by immunocytochemistry and flow cytometry in the spleens, lungs, and livers of mice, with measurable production of total human IgG and *T. gondii*-specific antibody in mouse serum. These mice were found to develop a fatal pneumonitis and toxoplasma encephalitis with dissemination of the parasite to multiple sites. Despite the greater severity of disease and parasite load in the lungs of unconditioned hu-PBL-engrafted mice in comparison to non-engrafted animals [7], preliminary analysis of mRNA for human β -actin in the lung, liver, and spleen indicated that human cells were not present in high numbers outside of the peritoneal cavity. It appeared that the IP route of infection may have interfered with the dissemination of the previously engrafted human cells or that the human cells have provided a reservoir of susceptible cells for infection and dissemination of the parasite. Conditioning by neutralization of the SCID mouse endogenous NK cells using γ -radiation and anti-asialo-GM1 (ASGM1) antibodies resulted in more prolonged engraftment by human cells, increased inflammatory scores, and worsened mortality when compared to non-engrafted SCID mice. Addition of IL-2 to the conditioning regimen resulted in similar histological findings to unconditioned hu-PBL SCID mice which were associated with high titre *T. gondii*-specific antibody and reversal of the adverse mortality seen in unconditioned animals. To analyze these possible confounding factors, an alternative route of infection, delivery of *T. gondii* PO by gavage, was investigated in this study.

Materials and Methods

Mice

Inbred C57BL/6, outbred Swiss-Webster and C.B-17 scid/scid (SCID) mice (female, 6–8 weeks of age) were ob-

tained from the Animal Resource Centre [Murdoch University, Perth, Western Australia (WA)]. SCID mice were housed in filter-top cages in a laminar-flow tissue culture hood with autoclaved bedding, food, and water.

T. gondii strains

The RH strain was obtained from Prof. A. Johnson (University of Technology, Sydney, NSW) and maintained by serial passage in the peritoneal cavity of Swiss Webster mice. Cysts of the DAG strain [8] were provided by Prof. J. S. Remington (Palo Alto Medical Foundation Research Institute, San Francisco, CA) and maintained by serial passage in C57BL/6 mice. Cysts were obtained by harvesting brains of mice at least 2 months after infection and purifying on a Percoll gradient as previously described [9, 10]. Bradyzoites were released from cysts by addition of 0.5 ml of a 1% trypsin solution in PBS to 2 ml of cysts. This was diluted immediately in 50 ml of PBS and centrifuged at 580 g for 10 min. The supernatant was discarded and the wash repeated twice. Bradyzoites were then counted using a hemocytometer. Mice were generally infected with 10⁴ bradyzoites.

Pre-transplant conditioning of SCID mice

Mice were given 2 Gy γ -radiation (Siemens Stabilipan, Germany) and 200 μ g IP polyclonal rabbit anti-asialo-GM1 (ASGM1) antibodies (Wako, Osaka, Japan) 1 day prior to PBL transplantation. Human PBL were isolated on Ficoll-Hypaque (Pharmacia LKB, Sweden) and 15–20 $\times$ 10⁶ PBL injected IP per mouse, in a volume of 0.2 ml. Polyethyleneglycol-conjugated human IL-2 (PEG-IL-2) (Chiron, Emeryville, CA) was reconstituted with sterile water, and 0.044 mg per mouse was administered IP with the PBL to expand numbers of human T-cells *in vivo* [2].

Donors

Informed consent was obtained from PBL donors and the study was approved by the Committee for Human Rights, University of Western Australia. The toxoplasma sero-positive donor had a symptomatic infection and an agglutination titre of 1 in 1280 by the direct agglutination test [11].

Detection of stage-specific products of toxoplasma

This was performed by RT-PCR and Southern blotting as previously described [7]. Tissues were snap frozen in liquid nitrogen, homogenized in 1 ml of RNAzol (Tel-Test, Friendswood, TX) and RNA isolated as described by the manufacturer. The RT reaction was performed with oligo dT(s) and AMV reverse transcriptase (Biotech International). The PCR primers (5'–3') and probes described

previously include: BAG1: which detects bradyzoites [12] [primers: agccatttccccgagtg, acttcacgctgattgttgctt (616 bp product size) and probe: tttgactgagcgagtgctcgg]; SAG1: which only detects tachyzoites [13] [primers: atgtcggttctgctgcacttc, tcacgcgacacaagctgcgtagagcc (1010 bp) and probe: ctcccactcttgcgtactcacc [14]; B1: which detects both forms of the parasite [15] [primers: ggaactgcacccgttcagag, tctttaagcgttcgtgggc (193 bp) and probe: ggcgaccaatctgcgaatacacc [16].

Human-specific primers and probes were tested against murine cDNA to ensure there was no homology and vice versa. Murine β -actin: [primers: tggaatcctgtg-gcatcatgaaac, taaaacgcagctcagtaacagtcg (362 bp, [17]). This housekeeping gene was used as a standard to ensure that cDNA concentrations were equal between samples. Human β -actin: [primers: aag aga tgg cca cgg ctg ctt, ggg gga caa aaa ggg gga agg, (941 bp product) and probe: ccg gcc cct cca tcg tcc acc].

PCR products were Southern blotted onto Hybond N+ nylon membrane (Amersham, England) using standard procedures [18]. Probes labeled with fluorescein (Operon) were added and hybridized at 42 °C overnight. Anti-fluorescein horseradish peroxidase conjugate (Amersham) was added at 1:500 for 1 h to detect the fluoresceinated probe.

Human IgG determination

Sera from transplanted hu-PBL SCID mice were tested for the presence of human IgG on a Beckman Array Analyser (Beckman Instruments, CA, USA) using the CSF protocol. Samples were diluted 1:5 in supplied buffer (Beckman). The lower level of detection was 10 mg/l of human IgG.

Fig. 1. Kaplan–Meier plot showing morbidity of *T. gondii*-infected SCID mice. SCID mice (5/group) were infected with ▲: 10^3 , ○: 10^4 , ■: 10^5 bradyzoites by the oral route and examined daily

Titration of *Toxoplasma*-specific antibody

Freshly lysed CHO cell cultures infected with the RH strain of *T. gondii* were prepared. Tachyzoites were counted and the pellet resuspended in 10 ml 6% formaldehyde solution in PBS for at least 16 h, then centrifuged at 900 g/10 min/4 °C and washed three times in PBS. The tachyzoites were suspended in alkaline buffer at a concentration of approximately $10\text{--}20 \times 10^6$ tachyzoites/ml. If CHO cells or cell debris were present, the solution was centrifuged 15 g/5 min, and the supernatant was collected. The agglutination test was based on the method reported by Desmonts and Remington [11]. Sera were serially diluted from 1:20 to 1:1280 in PBS and 50 μ l added to 50 μ l of 0.2 M 2-ME and 50 μ l of the *T. gondii* antigen preparation. The tray was mixed for 5 min and incubated at room temperature overnight. Agglutination was scored visually on a white light box.

Statistical analysis of mortality data

Data were calculated using StatView 5 application (Abacus Concepts, CA). Kaplan–Meier survival curves were constructed for each experimental group and differences in survival were assessed by generalized Wilcoxon's test [19].

Results

The study was designed to identify whether use of the physiological route of human infection (i.e. oral) resulted in a different outcome of infection when compared to IP infection using an avirulent human isolate of *T. gondii* [6]. This required an initial dose-ranging study to optimize the inoculation size. A comparison was then made of the effect of presence or absence of *T. gondii* infection on distribution of engrafted human cells. Finally, differences in distribution of human cells, human antibody, and parasites during the course of infections induced by different challenge routes (IP and PO) was studied to explain any observed differences in outcome.

Dose response of SCID mice after oral infection with *T. gondii*

SCID mice (5 per group) were conditioned by irradiation and anti-asialo GM₁ antibody treatment as previously described [6] and infected orally with 10^3 , 10^4 or 10^5 bradyzoites. The mice were examined daily and the morbidity results are shown in Fig. 1. The dose of 10^5 bradyzoites led to the consistent rapid demise of animals from infection from 20 to 26 days p.i. A dose of 10^4 bradyzoites led to a more delayed infection with mice succumbing to infection from 25 to 32 days p.i. The dose of 10^3 bradyzoites led to a slower pace of infection of animals which began to succumb from the 25th day p.i. with some long-term survivors

Table 1. The distribution of human β -actin in the tissues of uninfected and orally infected hu-PBL SCID mice

Group	Week post infection	Brain	Lung	Liver	Spleen	Number of mice with any positive organ	Total number of positive organs/group
Uninfected control	0	0/3	3/3	3/3	2/3	3/3	8/12
	1	0/3	2/3	2/3	1/3	2/3	5/12
	2	0/3	1/3	2/3	2/3	2/3	5/12
	4	0/3	2/3	2/3	3/3	3/3	7/12
PO infection	1	0/2	2/2	2/2	2/2	2/2	6/8
	2	0/3	1/3	1/3	1/3	1/3	3/12
	4	0/3	2/3	2/3	2/3	2/3	6/12

Conditioned hu-PBL SCID mice were transplanted with hu-PBL and tissues were collected for RT-PCR and Southern blotting for human β -actin expression at weekly intervals after infection. Control mice were uninfected.

observed. Hence, the 10^4 dose was chosen as optimal for subsequent experiments to attempt to demonstrate a significantly reduced or increased effect of interventions on the course of infection.

Engraftment of human cells in uninfected and orally infected hu-PBL SCID mice

The distribution of human cells was compared between uninfected animals and those that received *T. gondii* PO by gavage. One week after transplantation of hu-PBL from a toxoplasma seropositive donor, prior to infection, human β -actin was detected in most animals in the lung (3/3), liver (3/3) and spleen (2/3). After 1 week of infection (i.e. 2 weeks post hu-PBL transplantation) both animals were positive for human β -actin in lung, liver and spleen with 2/3 infected and 3/3 uninfected still positive for human β -actin in at least 2 of these organs by week 4 (Table 1).

Flow cytometry was also used to examine spleen cells from orally-infected animals and the percentage of human T cells were no higher (not shown) than those found previously in IP infected animals [6]. Engraftment of B lymphocytes was confirmed through the presence of human IgG in serum. Maximal titres were found at the latest time examined, 4 weeks p.i. with 940 mg/l ($\pm$ SD 227 mg/l) in uninfected animals and 520 mg/l ($\pm$ SD 485 mg/l) in infected animals (Fig. 2). Control mice, ($n = 4$) consisting of conditioned SCID mice, not transplanted with hu-PBL, did not contain detectable human IgG (< 10 mg/l).

The effect of the route of infection on the distribution of human cells

The ready detection of mRNA for human β -actin (used as a surrogate marker for engrafted human cells) in liver, spleen and lung following oral infection with bradyzoites of *T. gondii* differed from the lower levels seen in our previously reported analysis of mice infected IP with cysts [6]. This may have been due to the difficulty in preparing a reliable inoculum using low doses of cysts, so a direct comparison of the influence of the two routes on infection with bradyzoites was performed. Human β -actin mRNA was detected in the peritoneal cavity of animals infected with 10^4 bradyzoites by PO or IP routes (Table 2). At one week after IP infection human cells were detected in the lung and liver of only 1 of 2 animals, no human cells were detected by week 2 but they were present in the single surviving animal at week 3 p.i. (Table 2).

Fig. 2. Human IgG levels in the sera of hu-PBL SCID mice. Mice were transplanted with hu-PBL. One group of mice was infected (closed box) with 10^4 bradyzoites of *T. gondii* via the oral 1 week later. The other group consisted of uninfected (open box) mice. Serum was collected at weeks 1, 2, and 4 after infection (i.e. 2, 3, and 5 weeks after hu-PBL transplant). Human IgG was assayed on a Beckman Array Analyser using the CSF IgG protocol. Results are shown as the mean $\pm$ SD from three mice/group

Table 2. The expression of human β -actin in the tissues of hu-PBL SCID mice infected with *T. gondii*

Route of infection	Week post infection	Brain	Lung	Liver	Spleen	Peritoneal	Number of mice with any positive organ	Total number of positive organs/group
IP infection	1	0/2	1/2	1/2	0/2	2/2	2/2	4/10
	2	0/3	0/3	0/3	0/3	3/3	1/3	3/15
	3	0/1	1/1	1/1	1/1	1/1	1/1	4/5
PO infection	1	0/3	3/3	3/3	2/3	3/3	3/3	11/15
	2	0/3	3/3	3/3	2/3	3/3	3/3	11/15
	3	1/3	2/3	1/3	1/3	3/3	3/3	8/15

++ 2 animals did not survive to week 3 post-infection.

Conditioned hu-PBL SCID mice were infected with *T. gondii*, by IP or oral route, 1 week after hu-PBL transplantation. Tissues were collected for RT-PCR and Southern blotting for human β -actin expression at weekly intervals after infection, p.c. – peritoneal cavity.

As before, human β -actin could be detected in the lung, liver and spleen of most animals infected PO with *T. gondii*, 1 or 2 weeks previously. By 3 weeks p.i., 1/3 animals

contained human cells in the brain, liver, and spleen and 2/3 were positive for human β -actin mRNA in the lung. The proportion of animals with any organ positive for hu-

man β -actin was higher in the PO versus IP group (9/9 organs positive v 4/7). Similarly, the proportion of organs with detectable human β -actin was consistently higher (30/45 v 11/30).

Human IgG levels were greater at weeks 1 and 2 p.i. in animals infected by the PO route (an average of 160 mg/l $\pm$ SD 69 mg/l and 240 mg/l $\pm$ SD 84 mg/l, respectively) compared to IP infected animals (30 mg/l $\pm$ SD 42 mg/l and 120 mg/l $\pm$ SD 158 mg/l). At 3 weeks p.i., only one animal infected by the IP route survived, and it is interesting that this animal had a high level of IgG in the serum (1800 mg/l). One animal infected by the PO route had the same level of IgG in the serum (1800 mg/l), but the mean human IgG level of this group was lower (740 mg/l $\pm$ SD 930 mg/l). Control mice (conditioned SCID mice, not transplanted with hu-PBL) had no human IgG (<10 mg/l) in the serum.

Fig. 3. Human IgG levels in the sera of hu-PBL SCID mice infected with *T. gondii*. Infection by the oral or IP route. Mice were transplanted with hu-PBL and infected with 104 bradyzoites, 1 week later. Serum was collected at weeks 1, 2, and 3 after infection and human IgG assayed on a Beckman Array Analyser using the CSF IgG protocol. Results are for individual animals

Detection of *T. gondii*-specific IgG antibody

Sera were collected weekly from hu-PBL SCID mice (transplanted from a *T. gondii*-seropositive donor) infected with *T. gondii* either

Table 3. *T. gondii*-specific IgG antibody agglutination titres in hu-PBL SCID mice

Week post infection		<i>T. gondii</i> -specific IgG antibody titre	
		Oral infection	IP infection
Week 1	Mouse 1	< 1/20	1/1280
	Mouse 2	1/1280	1/1280
	Mouse 3	1/1280	n/d
Week 2	Mouse 1	< 1/20	1/1280
	Mouse 2	1/1280	1/20
	Mouse 3	1/1280	1/20
Week 3	Mouse 1	1/1280	< 1/20
	Mouse 2	< 1/20	died
	Mouse 3	< 1/20	died

SCID mice were transplanted with hu-PBL and infected with 10^4 bradyzoites of *T. gondii* by the oral or IP route, 1 week later. Serum was collected at weeks 1, 2, and 3 p.i. and *T. gondii*-specific IgG antibody measured using the agglutination test, n/d = not done.

by the PO or IP route and analyzed for the presence of *T. gondii*-specific IgG antibody. Similar high titres of *T. gondii*-specific IgG antibody were found in both IP and PO infected animals at weeks 1 and 2 but by week 3 2/3 IP-infected mice were dead, and the surviving mouse in this group did not have detectable antibody (Table 3).

Comparison of the distribution of *T. gondii* transcripts between IP- and PO-infected hu-PBL SCID mice

Animals infected by the IP route were clinically unwell by 17 days p.i. and two out of three animals died before the 3-week endpoint. In contrast, orally infected animals appeared well at 3 weeks p.i. At 1 week p.i., BAG1 transcripts, associated with bradyzoites, were detected in the liver of some IP- and PO-infected animals, and in the brain in one IP-infected animal (Table 4). By week 2 p.i., BAG1 transcripts were detected in the brain and lung after IP infection and in the lung after PO infection. BAG1 was detected in all organs of some animals at 3 weeks p.i. Inter-

estingly, one of three animals in the IP-infected group, and one of three animals in the PO-infected group had no evidence of infection in any organ at 3 weeks p.i. by mRNA analysis.

B1 transcripts, associated with both bradyzoites and tachyzoites, were not detected until 2 weeks p.i. in either IP- or PO-infected mice (not shown). At 2 weeks p.i., B1 was detected in the brain, lung and liver of some IP-infected mice and in the liver of orally infected mice. By week 3 p.i., B1 was present in the same PO-infected animals and tissues as those expressing BAG1.

SAG1 transcripts, which are produced by tachyzoites, were only detected in the lung of one PO-infected animal at 3 weeks p.i. (not shown). This animal also had B1 and BAG1 transcripts present in all tissues.

The distribution of human cells in these animals was shown previously in Table 2. It is apparent that in animals in which BAG1 was not detected (IP wk 1a, wk 2a, wk 3c and PO wk 1c, 2c, 3a) all, except one animal (IP wk2a), had human β -actin present in at least 2 other tissues apart from the peritoneal cavity.

Table 4. The expression of the BAG1 transcripts of *T. gondii* in hu-PBL SCID mice, after IP or oral infection

Route	Week p.i.	Brain	Lung	Liver	Spleen	Number of mice with any positive organ	Total number of positive organs/group
IP	1	1/2	0/2	1/2	0/2	1/2	2/8
	2	2/3	1/3	0/3	0/3	2/3	3/12
++	3	0/1	0/1	0/1	0/1	0/1	0/4
Oral	1	0/3	0/3	2/3	0/3	2/3	2/12
	2	0/3	2/3	0/3	0/3	2/3	2/12
	3	2/3	2/3	2/3	1/3	2/3	7/12

++ 2 animals did not survive to week 3 post infection.

Conditioned hu-PBL SCID mice were infected with 1×10^4 *T. gondii* bradyzoites by the IP or oral route, 1 week after hu-PBL transplantation. Tissues were collected for RT-PCR to detect expression of the BAG1 transcripts of *T. gondii* at weekly intervals after infection.

Discussion

These studies demonstrate that the route of administration of *T. gondii* significantly alters the pathogenesis of the infection in the hu-PBL SCID mice with the PO route not resulting in mortality from the DAG strain and being associated with more widespread dissemination of human cells, persistence of *T. gondii*-specific antibody and delay in appearance of markers of bradyzoites (BAG1 gene). This is in marked contrast to the consequences of infection by the IP inoculation route which results in early mortality and late disappearance of human cells and *T. gondii*-specific antibody which were readily detectable at earlier time points. This particular model using the PO route should be useful for dissecting the interdependence of chemotherapy and immunotherapy during toxoplasmosis in the immuno-compromised human in subsequent studies. Similar models have been shown to be useful in studies of human-specific organisms such as *Salmonella typhi* and HIV [20]. Unlike some other hu-PBL SCID models, our protocol does not appear to induce significant graft versus host disease (GVHD) [20]. These studies are not easy to achieve, however, as the expense of the animals (\$23 AUD/mouse at the time of these experiments) and the limited yield of cells from individual donors (sufficient to repopulate ~20 mice) allows only small experimental groups. Ideally experiments with increased mice per group would be used, perhaps by using donors undergoing cellular phoresis.

Human cells were detected in multiple tissues of conditioned hu-PBL SCID mice following infection by the PO route 1 week after human cell transfer. In contrast, after IP infection human cells were only readily detected in the peritoneal cavity by RT-PCR and probing. It was unclear whether oral infection encouraged cells to traffic from the peritoneal cavity (i.e. counter to the so-called Trojan horse hypothesis) [21], or cells dispersed before the infection became established because of its slower pace in the PO group. However, it was found that the presence of human cells in tissues of conditioned hu-PBL SCID was similar between uninfected or orally infected animals with human β -actin present in the lung, liver, and spleen of most animals. Thus, the presence of the parasite was not necessary for the engraftment of hu-PBL in the lung, liver, and spleen of the hu-PBL SCID mouse.

Since the parasite was administered 1 week after transplantation, by which time human cells could be found in multiple tissues in the uninfected SCID mouse, the failure to find human β -actin in IP-infected animals requires explanation. It is possible that human cells in the peritoneal cavity provided a reservoir of susceptible cells in which *T. gondii* multiplied and eventually destroyed the cells *in situ* thereby reducing the number of cells available for migration. Another possibility is that IP infection facilitated production of *T. gondii* infected immune cells which traffick to distant sites and accelerate the pace of infection, as has been described in mouse models [21]. Alternatively, if the presence of the parasite inhibited lymphocyte proliferation in the peritoneal cavity, by mechanisms which may

include processes such as STAT-3 suppression of macrophage activation [22], there may have been fewer human cells available to disseminate. Total human cells and percentage of T cells were observed to increase in number from 2 to 4 weeks after engraftment of uninfected SCID animals [6]. Evidence supporting suppression includes the fact that at 4 weeks after transplantation, the percentage of human T cells in the peritoneal cavity in uninfected animals by flow cytometry was 37%, higher than the level (5%) found in IP-infected animals [6].

It was found that the same dose of parasite given IP led to a more rapid and severe infection than seen in animals infected by the PO route. This difference in outcome may be due to a lower infectivity by the oral route with some of the inocula passing out through the feces. Alternatively, the IP site being the site of initial engraftment, the cells may have been destroyed *in situ* prior to distant trafficking. Thus, the more aggressive IP infection may have contributed to an increased destruction of human cells. It is interesting, therefore, that similar levels of human IgG were found in the serum of infected and uninfected animals, suggesting similar levels of B cell engraftment and activity. Since EBV-transformed human B cells can be infected by *T. gondii*, it is unlikely that the human B cells would have been selectively spared from infection.

Interestingly, the two mice with a high level of human IgG (1800 mg/ml) at 3 weeks p.i. also had human β -actin present in liver, lung, and spleen and *T. gondii* B1, BAG1, and SAG1 mRNA could not be detected in either animal. Cysts and occasionally tachyzoites of *T. gondii* were present in animals in which human β -actin mRNA was not detected. Therefore, it appears that when high levels of human engraftment are achieved, there is protection against *T. gondii*. The donor used in the currently reported study had a previous episode of lymphadenopathic toxoplasmosis and a higher *T. gondii*-specific agglutination titre than the donor used in the previous study [6]. It is therefore likely that this donor had a higher frequency of *T. gondii*-specific lymphocytes. These results were unexpected since in the original model, where cysts were delivered PI, the presence of hu-PBL led to the earlier appearance of mRNA for B1 in the brain and also greater amounts of SAG1 expression in the lung [6, 7].

Since neither human nor murine cells entirely eliminate the tissue cyst form of the parasite *in vivo*, it is likely that cysts do exist in the animal below the limits of detection by RT-PCR. Although RT-PCR and Southern blotting is a very sensitive method of detection, the amount of tissue analyzed is small (1 μ g). The sensitivity of RT-PCR and Southern blotting for parasite detection was found to be 1 bradyzoite by extraction of 1 μ g RNA from lysed cysts [7]. In studies of hu-PBL SCID mice 1 μ g of total tissue RNA was used for RT-PCR. Thus, the starting tissue used in sensitivity studies would contain substantially more parasite RNA.

In conclusion, the PO route of acute infection resulted in an enhanced engraftment of human cells in tissues of the SCID mouse compared to IP-infected animals. In addi-

tion, the use of the oral model resulted in a slower infection than in the IP model. It was noted that some animals which had high levels of human cell engraftment did not have detectable parasite tachyzoites or bradyzoites in brain, lung, liver, or spleen suggesting that control of toxoplasmosis may have occurred. We believe that these data reported above suggest that the PO infection model will be useful for more in-depth analysis of *in vivo* immune responses against *T. gondii*, as well as therapeutic studies of candidate drugs for treatment. Preliminary studies (data not shown) have already demonstrated *in vivo* production in hu-PBL SCID mice of *T. gondii*-mediated human IFN- γ production.

Acknowledgements

This work was supported by grant 950979 from the National Health and Medical Research Council of Australia. We thank Carla Smith for excellent assistance in the care of animals.

References

- Frenkel JK: Pathophysiology of toxoplasmosis. *Parasitol Today* 4, 273–278 (1988)
- Beaman M (2010): The role of immunomodulation in the management of infections of the CNS. In: *Inflammatory Diseases of the Central Nervous System*, eds Kilpatrick T, Wesslingh S, and Ransonhoff R, Cambridge University Press, Cambridge. pp. 221–244
- Johnson AM: Strain-dependent, route of challenge-dependent, murine susceptibility to toxoplasmosis. *Z Parasitenkd* 70, 303–309 (1984)
- McLeod R, Estes RG, Mack DG, Cohen H: Immune response of mice to ingested *Toxoplasma gondii*: a model of toxoplasma infection acquired by ingestion. *J Infect Dis* 149, 234–244 (1984)
- Liesenfeld O, Kosek J, Remington JS, Suzuki Y: Association Of Cd4(+) T cell-dependent, interferon-gamma-mediated necrosis of the small intestine with genetic susceptibility of mice to peroral infection with *Toxoplasma gondii*. *J Exper Med* 184, 597–607 (1996)
- Beaman MH, Remington JS, Meyer DJ: Human peripheral blood lymphocyte severe combined immunodeficiency (hu-PBL SCID) models of toxoplasmosis. *Immunol Cell Biol* 78, 608–615 (2000)
- Meyer DJ, Allan JE, Beaman MH: Distribution of parasite stages in tissues of *Toxoplasma gondii* infected SCID mice and human peripheral blood lymphocyte-transplanted SCID mice. *Parasite Immunol* 22, 567–579 (2000)
- Suzuki Y, Conley FK, Remington JS: Differences in virulence and development of encephalitis during chronic infection vary with the strain of *Toxoplasma gondii*. *J Infect Dis* 159, 790–794 (1989)
- Blewett DA, Miller JK, Harding J: Simple technique for the direct isolation of toxoplasma tissue cysts from fetal ovine brain. *Vet Rec* 112, 98–100 (1983)
- Huskinson-Mark J, Araujo FG, Remington JS: Evaluation of the effect of drugs on the cyst form of *Toxoplasma gondii*. *J Infect Dis* 164, 170–177 (1991)
- Desmonts G, Remington JS: Direct agglutination test for diagnosis of *Toxoplasma* infection: method for increasing sensitivity and specificity. *J Clin Microbiol* 11, 562–568 (1980)
- Bohne W, Gross U, Ferguson DJ, Heesemann J: Cloning and characterisation of a bradyzoite-specifically expressed gene (*hsp30/bag1*) of *Toxoplasma gondii*, related to genes encoding small heat shock proteins of plants. *Mol Microbiol* 16, 1221–1230 (1995)
- Burg L, Perelman D, Kasper LH, Ware PL, Boothroyd JC: Molecular analysis of the gene encoding the major surface antigen of *Toxoplasma gondii*. *J Immunol* 141, 3584–3591 (1988)
- Gazzinelli RT, Eltoun I, Wynn TA, Sher A: Acute cerebral toxoplasmosis is induced by *in vivo* neutralization of *tnf*- α and correlates with the down-regulated expression of inducible nitric oxide synthase and other markers of macrophage activation. *J Immunol* 151, 3672–3681 (1993)
- Boothroyd JC, Burg J, Nagel S, Perelman D, Kasper L, Ware P, Prince J, Sharma S, Remington J (1987): Antigen and tubulin genes of *Toxoplasma gondii*. In: *Molecular Strategies of Parasitic Invasion*, eds Agabian N, Goodman H, Nogueira N, Alan R, Liss Inc. New York. pp. 237–250
- Burg J, Grover CM, Pouletty P, Boothroyd JC: Direct and sensitive detection of a pathogenic protozoan, *Toxoplasma gondii*, by polymerase chain reaction. *J Clin Microbiol* 27, 1787–1792 (1989)
- Autenrieth I, Bucheler N, Bohn E, Heinze G, Horak I: Cytokine mRNA expression in intestinal tissue of interleukin-2 deficient mice with bowel inflammation. *Gut* 41, 793–800 (1997)
- Sambrook J, Fritsch E, Maniatis T (1989): *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press
- Dawson-Saunders B, Trapp RG (1990): *Basic and Clinical Statistics*, Appleton and Lange, San Mateo, CA
- Schultz L, Brehm MA, Garcia-Martinez JV, Greiner, DL: Humanized mice for immune system investigation: progress, promise and challenges. *Nature Rev Immunol* 12, 786–798 (2012)
- Courret N, Darche S, Sonigo P, Milon G, Buzoni-Gatel D, Tardieux I: CD 11c and CD11b-expressing mouse leukocytes transport single *Toxoplasma gondii* tachyzoites to the brain. *Blood* 107, 309–316 (2006)
- Butcher B, Kim L, Panopoulos AD, Watowich SS, Murray PJ, Denkers EY: Cutting edge: IL-10-independent STAT3 activation by *Toxoplasma gondii* mediates suppression of IL-12 and TNF- α in host macrophages. *J Immunol* 174, 3148–3152 (2005)