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Toxoplasma gondii infection can regulate the expression of tumour necrosis factor-α receptors on human cells *in vitro*

DORRA DEROUICH-GUERGOUR[1], DELPHINE ALDEBERT[1], INES VIGAN[2], EVELYNE JOUVIN-MARCHE[2], PATRICE N. MARCHE[2], DOMINIQUE AUBERT[3], PIERRE AMBROISE-THOMAS[1] & HERVÉ PELLOUX[1]

[1]Interactions Cellulaires Parasite-Hôte (EA.UJF 2940), Faculté de Médecine, Université J. Fourier, Grenoble, [2]Laboratoire d'Immunochimie, CEA-G/DBMS, INSERM U548, Université J. Fourier, Grenoble and [3]Laboratoire de Parasitologie (UPRES EA-2070), IFR-Biomolécules, Université de Reims, Reims, France

SUMMARY

The in vitro *regulation of tumour necrosis factor (TNF)-α receptors during* Toxoplasma gondii *infection of human MRC5 fibroblasts and human myelomonocytic THP-1 cells was investigated. Cells were infected with the virulent RH of* T. gondii. *TNFR membrane receptors were analysed by flow cytometry with biotinylated TNF-α. Shedding of the soluble form of TNFR1 and TNFR2 in cell culture supernatants was measured by enzyme-linked immunosorbent assay, and expression of mRNA production of TNFR1 and TNFR2 was analysed by quantitative real-time ploymerase chain reaction, 1 h after infection. In the MRC5 cell line,* T. gondii *infection did not induce any up- or down-regulation of membrane TNFRs, soluble TNFRs or mRNA of TNFRs. However, THP-1 cell infection with living parasites induced a significant soluble TNFR1 release by THP-1 cells after 1 h. We detected an approximately 50% up-regulation (*$P < 0{\cdot}01$*) of soluble TNFR1 in infected THP-1 cells compared to controls. No change in soluble TNFR2 levels was observed in the same conditions. Moreover, infection decreased the level of TNF membrane receptors, but had no effect on TNFR1 and TNFR2 mRNA levels. TNFR modulation by* T. gondii *infection,* in vitro, *depends on the cell type. Furthermore, our data suggest that living parasites control the shedding of the soluble form of TNFR1. This mechanism may influence the role of TNF-α in toxoplasmosis.*

Keywords *TNF-α receptors,* Toxoplasma gondii, *MRC5, THP-1, quantitative RT-PCR, flow cytometry, ELISA*

Correspondence: Dr Dorra Derouich-Guergour, Interactions Cellulaires Parasite-Hôte (EA.UJF 2940), Faculté de Médecine, Université J. Fourier Grenoble 1, Domaine de la Merci, 38706 Grenoble, France (e-mail: dorra.derrouich-guergour@ujf-grenoble.fr).
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INTRODUCTION

Toxoplasma gondii, an intracellular coccidian, has the ability to infect and propagate in virtually all nucleated host cells (1). It causes a worldwide infectious disease, toxoplasmosis. This infection is normally innocuous in individuals with an intact immune response (2) that results in a well-balanced interaction between the host and the parasite. However, in situations of compromised immune function, this balance is upset leading to life-threatening disease. This is the case of congenital infection in new-borns and the immunocompromised host, such as transplant recipients, AIDS patients and cancer chemotherapy patients (3,4).

Host resistance to *T. gondii* is associated with a polarized Th1 type cytokine expression pattern (5,6). Tumour necrosis factor (TNF)-α, in addition to a interferon-γ-mediated immune response, plays a major role in host resistance to toxoplasmosis (7–9).

TNF-α is a highly pleiotropic cytokine associated with a broad spectrum of biological effects (10). This cytokine elicits its biological functions through activation of two distinct cell surface receptors: TNFR1 (also known as p55, p60 or CD120a) and TNFR2 (also known as p75, p80 or CD120b). These two receptors belong to the TNF-NGF-receptor family. They transduce distinct signals and are present on virtually all cell types, including macrophages, lymphocytes and fibroblasts (11,12). Human TNFR1 and TNFR2 extracellular domains share 28% identity (12), while they display no homology in their intracellular domains (13). The lack of homology indicates that the two receptors probably use different intracellular signalling pathways when stimulated. TNFR1 is known to mediate most of the effects of TNF-α (14,15) and it has been postulated that the role of the TNFR2 is primarily to enhance signalling through the TNFR1 by recruiting and delivering TNF-α to lower affinity TNFR1 (14). The receptor expression can be modulated in response to various stimuli, such as cytokines, protein

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kinase, oncogenes and proteases, and thus represents one possible level of control of TNF-α responsiveness.

Both TNFR1 and TNFR2 have been shown to exist in soluble forms produced from membrane proteolytic cleavage (16) and act either as agonists or as inhibitors of TNF-α bioactivity (17,18). They are present in biological fluids, including serum, urine, ascite and synovial fluid, under both normal and pathological conditions (19–22).

Very few studies have been devoted to the role of TNFRs in *T. gondii* infection. *In vitro*, it has been reported that infection of murine macrophages induces down-regulation of surface TNFR which is dependent on the production of suppressive transforming growth factor β (23). *In vivo*, studies using gene-deficient mice have shown that TNF receptor signalling is not required for early control of *T. gondii* but is critical for the prevention of toxoplasma encephalitis later in infection (24,25).

We reported that TNF-α enhances *T. gondii* cyst formation in human MRC5 fibroblasts (26) and, in the present study, we examined the effects of *T. gondii* infection on the regulation of TNF receptors *in vitro* on two human cell lines at the mRNA, membrane protein and soluble protein levels.

MATERIALS AND METHODS

Cell culture

Human embryonic lung MRC5 diploid fibroblasts ATCC CCL 171 (BioMérieux, Marcy L'Etoile, France) were grown in 24-well plates on glass coverslips or in 25 cm^2 tissue culture flasks in Dulbecco's modified Eagle's medium supplemented with 2% synthetic serum Ultroser G (Life Technologies, Eragny, France), 25 mM Hepes, 4 mM glutamine, 500 IU/ml penicillin and 250 μg/ml streptomycin at 37°C in a 5% CO_2 humidified atmosphere, and were allowed to reach confluence.

The nonadherent human myelomonocytic THP-1 cells ATCC 202 TIB were grown in RPMI 1640 (Gibco BRL, Cergy Pointoise, France) supplemented with 100 IU/ml Penicillin, 100 μg/ml Streptomycin and 10% heat decomplemented (30 min at 56°C) fetal calf serum (FCS) (Bio-whittaker, Belgium). Cells were incubated at 37°C in 5% CO_2 humidified atmosphere. The cell density ranged between 1 and 5×10^5 cells/ml. Cells were harvested twice-weekly in 25 or 75 cm (2) cell culture flasks and were used when viability exceeded 95% by the trypan blue exclusion test.

Toxoplasma gondii strain and infection protocol

The virulent RH strain (Sabin 1941) was maintained in our laboratory by intraperitoneal passages in Swiss-OF1 mice (28). Tachyzoïtes were harvested from peritoneal ascitic fluid of Swiss mice (IFFA-Credo, L'Arbresle, France) infected 3 days earlier. For a multiplication cycle, parasites were passed *in vitro* on confluent MRC5 cells. Three days after infection, tachyzoites were collected from the culture supernatant (1200 ***g*** for 10 min), washed twice in phosphate-buffered saline (PBS) and counted in a Malassez cell. Their viability was assessed using acridine orange-ethidium bromide, and parasite suspensions with a viability > 95% were used.

MRC5 cells were used at confluence and THP-1 cells were used in the exponential growth stage (day 3). Cells were infected with a ratio of 10 parasites per cell, in fresh medium. The cultures were maintained at 37°C in saturated 5% CO_2 air and harvested 1 h after infection. Depending on the experiments, cells were stimulated with heat-killed tachyzoïtes (56°C for 50 min) (28) or D609-treated tachyzoïtes. Briefly, parasites were incubated for 2 h with 30 mg/ml D609, a specific inhibitor of phosphatidylcholine-specific phospholipase C, which was previously shown to be involved in parasite invasion and cyst formation (26,30). Pre-treated parasite suspensions with a viability < 95% were used.

Invasion assay

Tachyzoite invasion of target cells was assessed by microscopic examination. Cells were stained with DiffQuick (Baxter, Maurepas, France). One hour after infection, intracellular parasites were counted in 1000 cells on each coverslip (for MRC5 cells) or each well (for THP-1 cells).

Quantification of TNFR mRNA

Nucleic acid isolation

Total RNA was isolated from cells using the High Pure RNA isolation Kit (Roche Diagnostics, Meylan, France). Residual DNA was removed by resuspending the RNA in 100 μl DNAse I buffer and incubating with 20 U RNAse-free DNAse I for 20 min at room temperature, followed by elution from the spin column in a volume of 50 μl.

Generation of cDNA

Aliquots of 15 μl RNA was reverse transcribed for 60 min at 37°C using 50 IU of Strata-Script™-reverse transcriptase (RT) and oligo-(dt) as primer (ProSTAR™ First-Strand RT-polymerase chain reaction (PCR) Kit, Clontech, Saint Quentin en Yvelines, France).

PCR assay for TNFR1 and TNFR2 gene transcripts

The quantification of TNFR transcripts in MRC5 and THP-1 was carried out with the LightCycler PCR system (Roche Diagnostics) using the DNA binding SYBR Green I

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dye for the detection of PCR products. According to general procedure previously described (31), the glyceraldehyde-3-phosphate deshydrogenase (G3PDH) gene was used as reference. Relative concentrations of TNFR1, TNFR2 and G3PDH transcripts were determined by comparison to a DNA external standard. TNFR2 and G3PDH primers were selected using DNA Star Primer Select Software (Madison, WI, USA). TNFR2 (product size 158 pb, annealing temperature 63°C), upstream primer: 5′-TCC CAA GCC AGC TCC ACA ATG-3′, downstream primer: 5′-AGG GGC AGG GGC TTC TCT TC-3′. G3PDH (product size 137 pb, annealing temperature 59°C), upstream primer: 5′-AGC AAT GCC TCC TGC ACC ACC AAC-3′, downstream primer: 5′-CCG GAG GGG CCA TCC ACA GTCT-3′. TNFR1 primers were commercialized by Clontech (product size 586 pb, annealing temperature 60°C), upstream primer: 5′-ATT TGC TGT ACC AAG TGC CAC AAA GGA ACC-3′, downstream primer: 5′-GTC GAT TTC CCA CAA ACA ATG GAG TAG AGC-3′.

DNA standards were generated by PCR and isolated from 0·5% agarose gel using QIAEX II Agarose Gel extraction Kit (Qiagen, Courtaboeuf, France). The amount of extracted DNA was quantified by spectrophotometry and expressed as copy number. Serial dilutions were used to generate each standard curve.

The analysis on the LightCycler was performed in a reaction volume of 20 µl using 1 × LightCycler-DNA master SYBRGreen I (Roche Diagnostics), 2 mM $MgCl_2$, 0·5 µM of each primer and 2 µl of cDNA matrix. A hot start procedure was employed by the addition of anti-Taq DNA polymerase (0·07 µM) to the amplification reaction mixture (Clontech, Heidelberg, Germany).

After 2 min of denaturation at 95°C, the reaction was cycled 40 times for 5 s at 95°C, 10 s or 5 s at the annealing temperature for G3PDH/TNFR2 and TNFR1, respectively, and 7 s or 24 s for G3PDH/TNFR2 and TNFR1, respectively.

The product specificity was determined by melting curve analysis as described in the LightCycler handbook. As an additional control of specificity, PCR products were removed from glass capillaries by reverse centrifugation, then size fractionated on 2% agarose gel and visualized under ultraviolet light. The amount of transcript in each sample is given as copy number. The results are expressed as ratio of TNFR1 or TNFR2 transcripts to G3PDH transcripts (× 1000). Yields of amplification for TNFR1, TNFR2 and G3PDH were in the same range.

Expression of membrane TNFRs

Expression of TNFRs was carried out by flow cytometry using human TNF-α biotin conjugate (Fluorokine™ R&D Systems Europe Ltd, Abingdon, UK). Adherent MRC5 cells were detached from plates with 0·05% ethylenediaminetetraacetic acid in PBS. After several washes with ice cold PBS (5 min, 500 ***g*** at 4°C), 10^5 MRC5 or THP-1 cells were incubated for 1 h at 4°C with the biotinylated cytokine which in turn binds to cells via cell surface receptors. The cells were then directly incubated for 30 min at 4°C with avidin-fluorescein, which attaches to the receptor-bound biotinylated cytokine. Cells expressing the specific TNFRs are fluorescent, and the mean of fluorescence intensity is directly proportional to the density of the receptors. Unstained cells were washed and treated in similar manner to measure the level of autofluorescence.

The specificity of staining was assessed using a protein (soybean trypsin inhibitor) biotinylated to the same degree as the TNF-α, and an anti-human TNF-α blocking antibody mixed with TNF-α biotin (both included in the Fluorokine™ kit).

The cells were stored on ice until analysis by FACS Calibur flow cytometer, using CellQuest software (Becton Dickinson, Pont-de-Claix, France). Measurements were based on 10 000 cells. Debris of cells were excluded by the conventional scatter gating.

Expression of soluble TNFRs

Soluble TNF receptors were detected using a specific enzyme-linked immunosorbent assay (ELISA) (Quantikine R&D Systems) according to the manufacturer's instructions. Culture supernatants were harvested after 1 h of culture, then centrifuged (5 min at 500 ***g***) and stored at –20°C until analysed. Values were calculated from a standard curve based on freshly prepared dilutions. Cell stimulated with phorbol 12-myristate, 13 acetate (PMA) 10^{-6} M were used as a positive control. Results were expressed as pg/ml. The sensitivity of the assay was 1·5 pg/ml. Each sample was peformed in duplicate and values were derived from a standard curve.

Statistical analysis

All experiments were performed three times or more. Statistical analysis was performed by Student's *t*-test and by analysis of variance. $P < 0{\cdot}05$ was considered statistically significant.

RESULTS

T. gondii infection reduced TNF membrane receptors on THP-1 cells and not on MRC5 cells

To analyse membrane receptors expression, we employed flow cytometry. No changes were seen regarding the level of

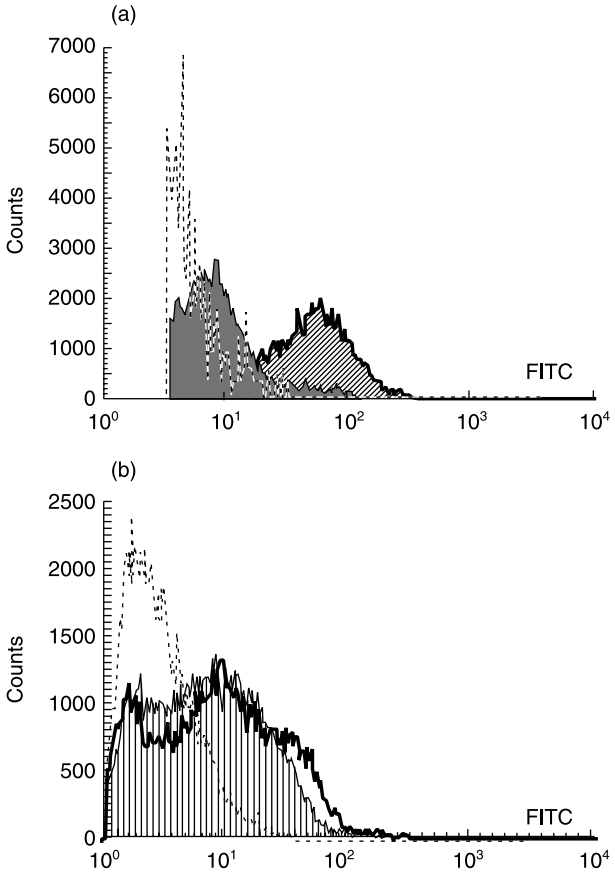

Figure 1 Expression of surface TNF-α receptors by uninfected and infected THP-1 cells (a) and MRC5 cells (b) with *T. gondii* (flow cytometry analysis). Representative histograms of the fluorescence intensity of THP1-associated TNF-α biotin are shown. Cells exposed to the avidin-fluorescein isothiocyanate (FITC) only were used as negative controls. The data presented are representative of six (a) and three (b) experiments performed. ---, Negative control; ▨, THP-1; ■, THP-1 + RH; □, MRC5; ▥, MRC5 + RH.

membrane TNFRs after infection of MRC5 with *T. gondii* (Figure 1b). However, the capacity of THP-1 cells to bind TNF-α biotin decreased after exposure to RH infection. As can be seen in Figure 1(a), the fluorescence intensity decreased in infected THP-1 cells, suggesting that these cells had a lower density of receptors per cell compared to uninfected cells.

The determination of the tachyzoite invasion rate showed no significant difference between the MRC5 and THP1 cell lines (Table 1).

T. gondii infection induces no modification in MRC5 cells, but induces release of sTNFR1 and not sTNFR2 in THP-1 cells

The effect of *T. gondii* infection of THP-1 and MRC5 cells on the release of the two soluble TNF-α receptors was determined by measuring the level of each soluble receptor in 1-h culture supernatants. While no difference in soluble TNFR levels between infected and native MRC5 was noted, a 50% increase ($P < 0{\cdot}05$) in the soluble TNFR1 released by infected THP-1 cells was found. Interestingly, no significant increase in the release of soluble TNFR2 was observed in these infected cells compared to controls (Figure 2a–c).

PMA treatment results in an approximatly 2·5–3-fold increase of the TNFR released by both cell lines ($P < 0{\cdot}001$).

T. gondii infection does not regulate TNFR mRNA in THP-1 and MRC5 cells

The enhanced shedding of sTNFRs could be from either preformed TNFRs or from neo-synthetized and secreted proteins. Consquently, real-time PCR detection by Light-Cycler was used to compare mRNA of TNFR1 and TNFR2 on unstimulated and *T. gondii* infected cells.

Our results showed that mRNA of both TNF receptors was detectable in the two untreated cell lines. As shown Figure 3(a,b), *T. gondii* infection did not affect mRNA expression of the two TNFRs.

No modulation after stimulation of THP-1 cells by heat-killed tachyzoïtes and D609-pretreated tachyzoïtes

Stimulation of THP-1 cells with heat-killed parasites did not change the basal level of soluble TNFR1, nor the number of membrane TNFRs. Figure 4 shows that parasites pretreated

Table 1 Effect of D609 on *T. gondii* (RH strain) penetration

	Tachyzoites not pretreated before infection	Heat-killed tachyzoites	Tachyzoites pretreated with D609 before infection
Infected MRC5 cells per 1000 cells	261 ± 25·51	–	–
Infected THP-1cells per 1000 cells	233 ± 12·58	0	75 ± 13·22*

Cell cultures were incubated for 1 h with tachyzoites of the RH strain of *T. gondii*. Data represent the number of cells harbouring the tachyzoites, as counted by microscopic analysis. Results are expressed as mean values from three independent experiments ± SD. *Significantly different ($P < 0{\cdot}05$).

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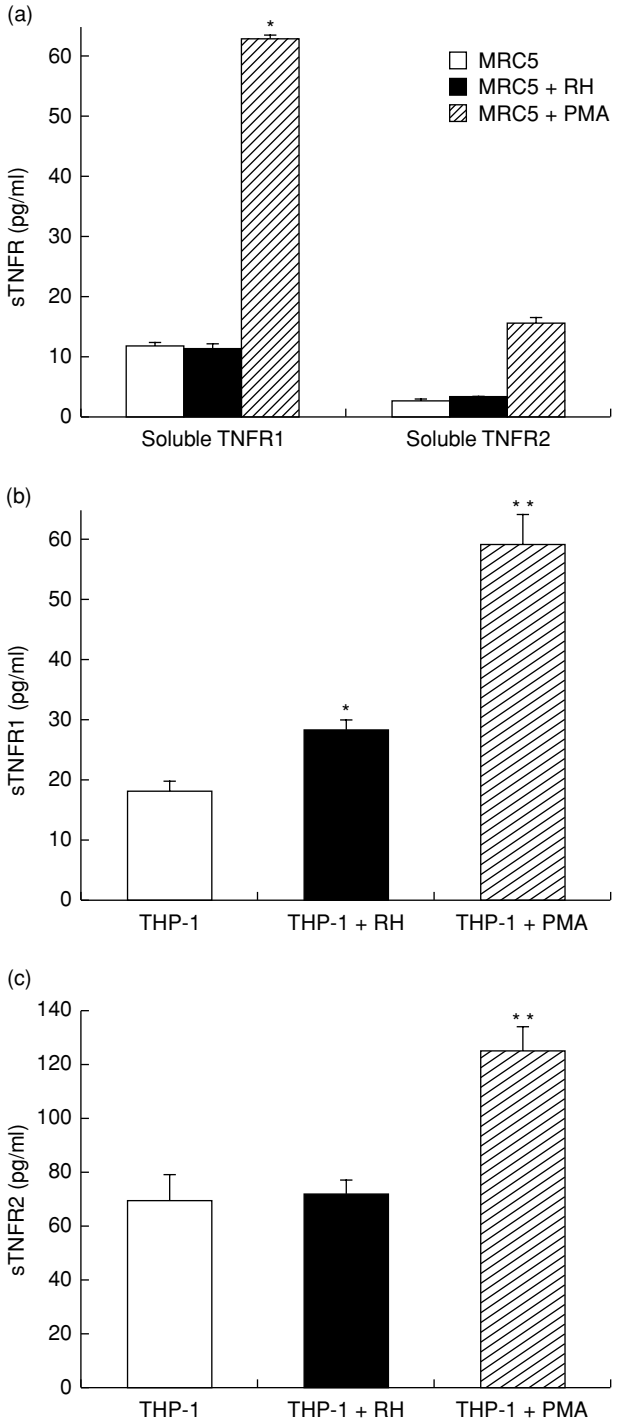

Figure 2 (a) Effect of *T. gondii* infection on the release of TNFRs by MRC5 cells.Aliquots of the culture medium of control cells, *T. gondii* infected cells and PMA stimulated were collected and tested for sTNFR1 and sTNFR2 as described in Materials and methods. Results are from one representative experiment out of three performed and are expressed as mean ± SD for triplicates. $*P < 0{\cdot}001$. (b) Effect of *T. gondii* (RH strain) infection on the release of TNFRs by THP-1 cells. Aliquots of the culture medium of THP-1 control cells, infected cells with *T. gondii* and cells stimulated with PMA for 1 h were collected and tested for sTNFR1 as described in Materials and methods. Results are from one representative experiment out of six performed and are expressed as mean ± SD for triplicates. In the six experiments, the increase of the soluble TNFR1 levels ranged from 38·6% to 62·7%. $*P < 0{\cdot}05$; $**P < 0{\cdot}001$. (c) Effect of *T. gondii* (RH strain) infection on the release of TNFRs by THP-1 cells. Aliquots of the culture medium of THP-1 control cells, infected cells with *T. gondii* and cells stimulated with PMA for 1 h were collected and tested for soluble TNFR2 as described in Materials and methods. Results are from one representative experiment out of six performed and are expressed as mean ± SD for triplicates. $**P < 0{\cdot}001$.

with D609 before infection did not induce the increase in soluble TNFR1 and the decrease in membrane TNFRs observed with nonpretreated tachyzoites. Invasion assays were performed in parallel and confirmed that, under our experimental conditions, the pretreatment of parasites with D609 before infection significantly reduced the penetration of *T. gondii* in THP-1 cells ($P < 0{\cdot}05$) (Table 1).

DISCUSSION

Since the modulation of cytokine-specific receptors expression represents an essential element of cytokine responsiveness, it was important to examine the effect of *T. gondii* infection on the cytokine receptors to better undrestand the mechanisms involved in *T. gondii* infection physiopathology. Furthermore, we and others have demonstrated that MRC5 and THP-1 cells lines constitutively express both TNFRs (32,33).

In the present study, we provide evidence that *T. gondii* infection of THP-1 cells modulates TNFR expression, but MRC5 fibroblast infection does not. Therefore, TNFR modulation by *T. gondii* infection may depend upon the human cell type used for *in vitro* infection. The difference observed between the two cell lines was not related to variation in the invasion rate.

Using flow cytometry, *T. gondii* infection was found to down-regulate the number of surface TNFRs on THP-1 cell line. The reduction in TNFR expression in THP-1 infected cells appears to affect the whole population, whereas only 25% of the cells are infected. This might be explained by a potential release of mediators by the infected cells, which would explain the decrease in the expression of TNF membrane receptors in noninfected cells.

We further show that this down-regulation was concomitant with a selective increase in the release of TNFR1 and not the TNFR2 in the culture supernatants. Enhanced TNFR release seems not to be due to TNFR1 formation *de novo* but probably to cleavage of membrane receptors. This is supported by the shedding of soluble TNFR1 not being

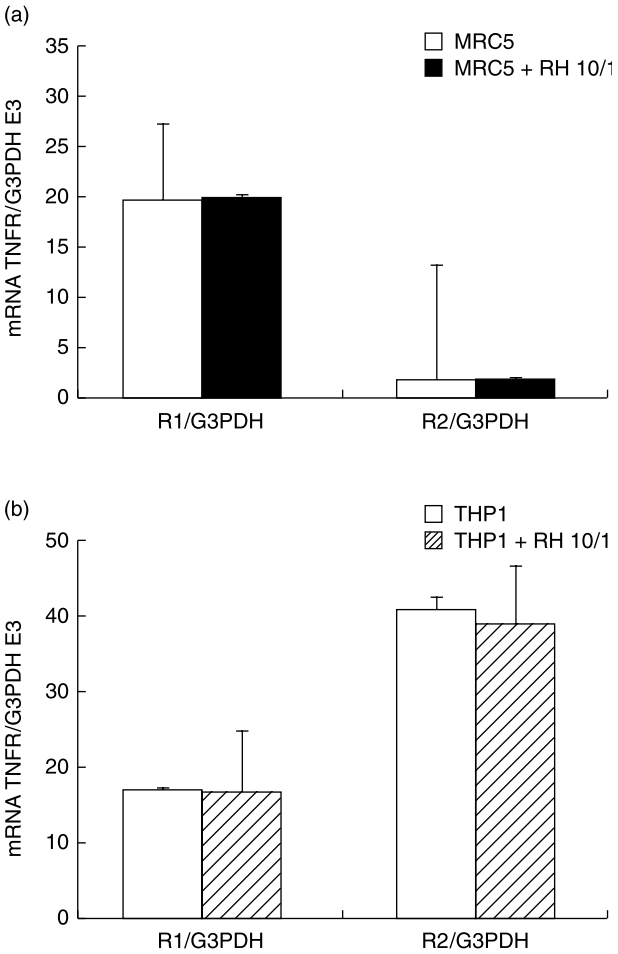

Figure 3 (a) Quantification of TNFR1 and TNFR2 RNA after *T. gondii* MRC5 infection by LightCycler.Total RNA from infected and uninfected cells were isolated after 1 h of culture and subjected to quantitative PCR after a reverse transcription step. Results are expressed as a ratio corresponding to TNFR cDNA copy/G3PDH cDNA copy number (× 1000). Data are mean ± SD values from three experiments. (b) Quantification of TNFR1 and TNFR2 mRNA after *T. gondii* THP-1 infection by LightCycler. Total RNA from infected and uninfected cells were isolated after 1 h of culture and subjected to quantitative PCR after a reverse transcription step. Results are expressed as a ratio corresponding to TNFR cDNA copy/G3PDH cDNA copy number (× 1000). Data are the mean ± SD values from three experiments. $P > 0{\cdot}5$.

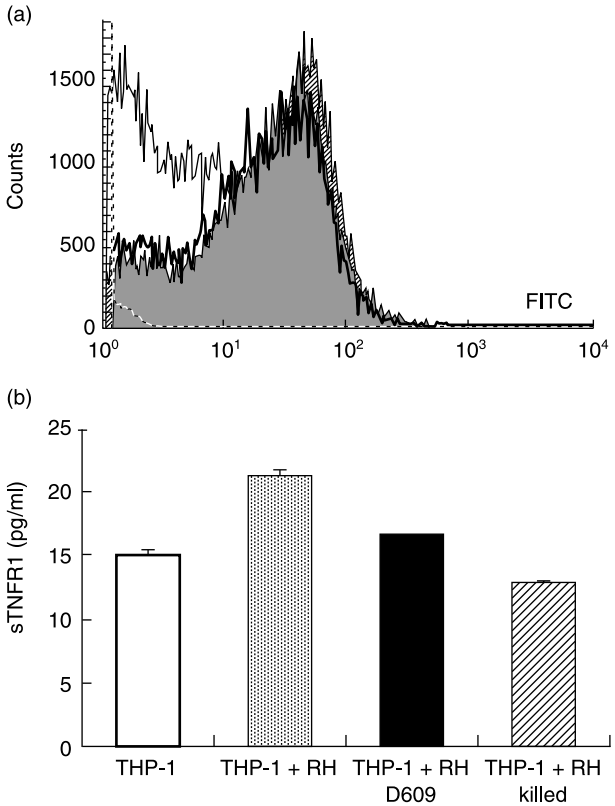

Figure 4 Role of the tachyzoites penetration in the regulation of surface TNF-α receptors expression and the release of TNFR1 by THP-1 cells. (a) Flow cytometry analysis. Representative histograms of the fluorescence intensity of THP-1-associated TNF-α biotin are shown. The data presented are representative of three experiments performed. —, Negative control; □, THP-1; □, THP-1 + RH; ▨, THP-1 + killed RH; ■, THP-1 + D609 RH. (b) Release of TNFR1 by THP-1 cells. Aliquots of the culture medium of THP-1 control cells, infected cells with *T. gondii* (pretreated or not) for 1 h were collected and tested for sTNFR1 as described in Materials and methods. Data are from one representative experiment out of four performed and are expressed as mean ± SD for triplicates. $*P < 0{\cdot}01$.

parallel to any change in TNFR1 mRNA transcription levels in THP-1 cells as detected by real time quantitative RT-PCR. Furthermore, all agents that down-regulate the surface TNF receptors appear to act through the shedding of the receptors (34).

The infection is associated with an up-regulation of the release of TNFR1 and not TNFR2. However, the protein kinase C activator, PMA, increases both TNFR1 and TNFR2 shedding in our experimental system, as well as in other systems (35,36). Thus, the differential regulation of TNFR shedding following *T. gondii* infection and PMA epxosure may reflect different enzymatic mechanisms by which the extracellular domain of the receptor is shed. This hypothesis is supported by the suggestion that a serine protease is involved in the spontaneous and PMA-induced cleavage of the two receptors from THP-1 cells (37). It is possible that shedding of TNFR1 during THP-1 *T. gondii* infection occurs by a mechanism different from the one induced by PMA. Furthermore, in U937 histiocytic lymphoma cell line, Higuchi & Aggarwal (38) reported that TNF-α induces sTNFR2 release and TNFR1 internalization.

Thus, our results indicate that TNFR1 and TNFR2 are independantly regulated in human THP-1 cells. This

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observation confirms and extends other reports. It has been shown that TNF-α and interleukin-1 regulate selectively TNFR2 in human fibroblasts (39), and the same selective regulation has been noted during activation of human B lymphocytes (40) and in cultured rat astrocytes (41).

The majority of TNF-dependent functions have been attributed to signal transduction via TNFR1 and, in contrast, a minor accessory role has been proposed for TNFR2, which many have a ligand passing role (12).

Several reports have shown that the signal through the TNFR1 is necessary for most biological functions of TNF-α *in vitro* and *in vivo* (14,15,42) and, in some systems, it has been demonstrated that internalization of the receptor is essential for its function (43). Signalling through this receptor was also shown to contribute to host defense against a number of micro-organisms by the use of gene-deficient mice in TNFR1 (44–46).

Here, we demonstrate that *T. gondii* infection of THP-1 cells induces a decrease in the number of surface membrane TNFRs. Similar observations have been reported with murine peritoneal macrophages and murine splenic macrophages in response to *T. gondii* and *Mycobacterium avium* infections (23,47).

In our model of THP-1 cells, the down-regulation of membrane TNFRs is significant in the infectious process because TNF-α is involved in the invasion of these cells by *T. gondii* (48). The down-regulation of membrane TNFRs and the appearance of soluble TNFR1 following infection could negatively affect TNF activity on these cells. It was recently shown that TNF-α activity was inhibited by local application of soluble TNFRs in an animal model of experimental periodontitis (49).

Although soluble TNFRs are considered as natural inhibitors of TNF-α activity (50,51), they may also potentiate TNF-α activity by stabilizing the trimeric structure of physiological TNF (17,18). In our model, we cannot verify the functional system of sTNFR1 by performing bioassays because THP-1 infected cells do not produce TNF-α in these culture conditions (48). Of particular interest is the finding that THP-1 cells produced soluble TNFRs in a TNFR1/TNFR2 ratio of 1 : 3·8 and also that *T. gondii* induced selective release of TNFR1 by these cells. Soluble TNFR1 is five to 30 times as potent as TNFR2 *in vitro* and is a better inhibitor of TNF-α activity *in vivo* (50,52).

We postulate that soluble TNFR1 might compete with cell surface TNFRs and thus act as an antagonist so that TNF-α binding is down-regulated. Our findings may also explain why *T. gondii* does not induce TNF-α release in these human cells, contrary to other microorganisms such as *Plasmodium falciparum* and *Legionella pneumophila* (53,54). The cytokine produced might be rapidly cleared from the medium after binding to soluble TNFR1.

To determine if the penetration of *T. gondii* in THP-1 cells is involved in the regulation of TNFRs observed, we stimulated cells with heat-killed tachyzoites and D609-pretreated tachyzoites, as specific inhibitor of PC-PLC (26,55). Heat-killed and D609-pretreated tachyzoites, which do not penetrate into cells, did not decrease the number of surface TNF receptors and did not increase the level of soluble TNFR1 in the culture medium. Thus, the active penetration of tachyzoites appears to be important in triggering TNF-α receptor regulation.

Although the role of TNF-α in the immune response to *T. gondii* is well documented, it is also known that the TNF-α effect *in vitro* is dependent on the model used (human or murine) and also on the nature of the human host cell involved (48,56,57). In this study, we suggest two aspects of survival of the parasite in human cells *in vitro*, involving the imbalance between TNF-α and its receptors. Our hypothesis is that somatic fibroblast cells (MRC5), after infection, may contribute to the recruitment of monocyte cells (THP-1) by secreted monocyte chemotactic protein-1 (30). Infection of THP-1 cells induces selective release of sTNFR1 and thereby reduction in the bioactivity of TNF-α. These data suggest that this mechanism may alter the mode of response of the host cells (MRC5 and THP-1) to the effects of TNF-α and favour the infection.

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