

Interaction between Human Immunodeficiency Virus and *Toxoplasma gondii* Replication in Dually Infected Monocytoid Cells

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THP-1 monocytoid cells, either not infected or chronically infected with human immunodeficiency virus type 1 (HIV-1), were challenged with *Toxoplasma gondii*. Parasitic growth, as assessed by trophozoite counting and measurement of supernatant p30 membrane antigen, was similar in HIV-infected and noninfected THP-1 cells. Also, *T. gondii* did not affect HIV replication. These experiments therefore failed to demonstrate any interaction between HIV-1 and *T. gondii* replication in concurrently infected monocytoid cells.

Toxoplasma encephalitis, which has emerged as one of the most frequent opportunistic infections in patients with AIDS, is usually consecutive to the reactivation of a previously latent infection in the brain (8). The pathogenesis of this reactivation is still poorly understood, but it is thought to be related in part to a decrease in cellular immune responses to the parasite. Indeed, the production of gamma interferon, mainly produced by CD4 T cells, is impaired in patients with AIDS (12), and this cytokine has been reported to play a critical role in the prevention of toxoplasmic encephalitis by activating intramacrophagic killing of the parasite (14). Also, defects in monocyte functions following human immunodeficiency virus (HIV) infection and direct interactions between HIV and *Toxoplasma gondii* might be involved in the pathogenesis of this parasitic infection (1). Monocytes are also the major target of both pathogens in the brain, and coinfection of these cells with the two pathogens is likely to occur in vivo (3, 5). We therefore investigated the interaction between HIV and *T. gondii* replication in cells of the monocyte-macrophage lineage.

The monocytoid cell line THP-1, derived from a patient with monocytoid leukemia (15), was used as a model. Cells were obtained from the American Type Culture Collection (TIB 202) and grown in supplemented RPMI 1640 medium as described previously (11). Cells were free of mycoplasma contamination. The HIV type 1 (HIV-1) IIIB strain (4), obtained from R. Gallo (National Cancer Institute, Bethesda, Md.), was propagated in THP-1 cells (11). Viral infection was monitored by supernatant reverse transcriptase (RT) activity (6), and detection of viral antigens was by the indirect immunofluorescence assay, using an anti-HIV p24 monoclonal antibody (Clonatec, France), and flow cytometry with a fluorescence-activated cell sorter (Becton Dickinson) (10). Chronically HIV-infected THP-1 cells, harvested 3 months after initial infection, were used in this study. More than 93% of these cells were stained by the anti-HIV p24 monoclonal antibody, and RT activity in the supernatant was $>10^6$ cpm/ml (11). Cell growth and viability (as determined by trypan blue exclusion) of these chronically

HIV-infected THP-1 cells were similar to those of noninfected cells (11).

THP-1 cells (5×10^4 per well in 96-well plates), chronically HIV infected or not infected, were then challenged with the virulent RH strain of *T. gondii*. The tachyzoites were collected from the peritoneal cavities of mice infected 3 days previously and resuspended at the desirable concentration in supplemented RPMI 1640 medium. Cultures were then incubated at 37°C in 5% CO₂. Preliminary experiments were performed to determine the optimal *T. gondii*/cell ratio of infection and the duration of the experiment. We chose two ratios of infection, 1:1 (one *T. gondii* parasite/one cell) and 1:2 (one *T. gondii* parasite/two cells), which allowed good viability of the coinfecting cultures for at least 4 days. Three independent experiments were performed. The growth of THP-1 cells, chronically HIV infected or not infected, was similar following infection with *T. gondii* (data not shown). Cell viability, as determined by trypan blue exclusion, remained above 90%.

Two different methods were used for monitoring *T. gondii* growth in cell culture. A direct counting of trophozoites was performed every day with a 50- μ l sample of the culture well under a phase-contrast microscope. Each measure was performed in quadruplicate. Also, measurement of *T. gondii* p30 antigen was performed in culture supernatants by enzyme-linked immunosorbent assay (ELISA). Briefly, culture plates were heated for 30 min at 56°C to inactivate HIV-1 and then kept frozen at -80°C until the assay. Supernatants were then assayed for p30 antigen by using an immunocapture ELISA. For that purpose, 96-well plates were sensitized, 2 h at 37°C and then overnight at 4°C, with 100 μ l of anti-toxoplasma rabbit immunoglobulin G antibodies (2) diluted 1/200 in carbonate buffer (pH 9.6). The ELISA plates were then saturated with gelatin (0.5%) in carbonate buffer for 1 h at 37°C. A 100- μ l portion of supernatant was added to each well, and the plates were incubated for 2 h at 37°C. Then 100 μ l of a 1/100 dilution of a peroxidase-labeled monoclonal antibody directed against the p30 membrane antigen of *T. gondii* (Diagnostics Pasteur, Narnes, France) was added. After 2 h at 37°C, 200 μ l of the substrate *o*-phenylenediamine was added to each well over 30 min. Photometric readings were performed at 492 nm. The results were expressed as the mean optical densities obtained from infected culture wells

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minus the mean optical densities obtained from uninfected controls (16 replicates were used in each experiment).

The growth of *T. gondii* in THP-1 cells, as assessed by trophozoite counting from days 1 to 4 of the coinfection, was not affected by a previous infection of the cells with HIV-1 (Fig. 1A; $P > 0.2$). However, starting on day 2 following *T. gondii* infection, the number of tachyzoites was significantly higher if a ratio of infection of one *T. gondii* parasite/cell was used compared with a ratio of one parasite/two cells (Fig. 1A; $P < 0.002$). These data were confirmed by measuring *T. gondii* p30 antigen with the ELISA as a marker of parasitic growth (Fig. 1B). No significant difference was observed in p30 antigen levels between HIV-infected or uninfected THP-1 cells from days 1 to 4 of the coinfection ($P > 0.3$). However, the p30 antigen level was significantly higher when a high inoculum of *T. gondii* (one parasite per cell) was used for infection compared with a low inoculum (one parasite per two cells). Moreover, a good correlation ($r = 0.96$) was found between trophozoite counting and p30 antigen levels.

To study the effect of *T. gondii* infection on HIV-1 replication in chronically HIV-infected THP-1 cells, RT activity assays were performed as follows: virus was concentrated from 50 μ l of cell-free supernatant, and RT activity was measured on days 1, 2, 3, and 4 following coinfection with *T. gondii* or mock infection (culture medium alone). Each experiment was done in triplicate, and three different experiments were performed. HIV-1 replication in chronically infected THP-1 cells, however, was not significantly affected by concurrent infection with *T. gondii* (Table 1; $P > 0.07$).

In this study, therefore, we have investigated a direct interaction between HIV-1 and *T. gondii* at the cellular level, following replication of each pathogen in concurrently infected THP-1 monocytoid cells. Infection of THP-1 cells with *T. gondii* was optimized to produce a concurrent infection of most THP-1 cells. For that purpose, THP-1 cells were chronically infected with HIV-1, with more than 93% of the cells infected, and then superinfected with *T. gondii*. During the 4 days of each experiment, the growth of THP-1 cells was similar following *T. gondii* infection, whether or not the cells were infected with HIV. When *T. gondii* replication was assessed by either trophozoite counting or measurement of p30 antigen, no significant difference was observed between HIV-infected and uninfected THP-1 cells. This is contrary to the results reported by Ikuta et al. (7), in which amplified replication of *T. gondii* was observed in persistently HIV-infected MOLT-4 cells. The discrepancy between our results and theirs could be related to the cell type we used, since the same viral strain of HIV was used. MOLT-4 cells are lymphoid cells, and THP-1 cells are monocytoid cells, which seem more relevant to the pathogenesis of *T. gondii* encephalitis, since lymphocytes are rarely seen in the brain. Our results are in agreement with those of Reed et al. (13), however, who used monocyte-derived macrophages as the target cell. Also, no effect of *T. gondii* infection on HIV replication was demonstrated in chronically HIV-infected THP-1 cells, similar to the data obtained by Ikuta et al. (7) with MOLT-4 cells coinfecting with HIV-1 IIIB.

In conclusion, the experiments we performed do not support a direct interaction between *T. gondii* and HIV replication in monocytoid cells. Although these results do not preclude such an interaction in another cell type or with other HIV or *T. gondii* strains, we are tempted to put our results together with those of Meylan et al. (9) and Reed et

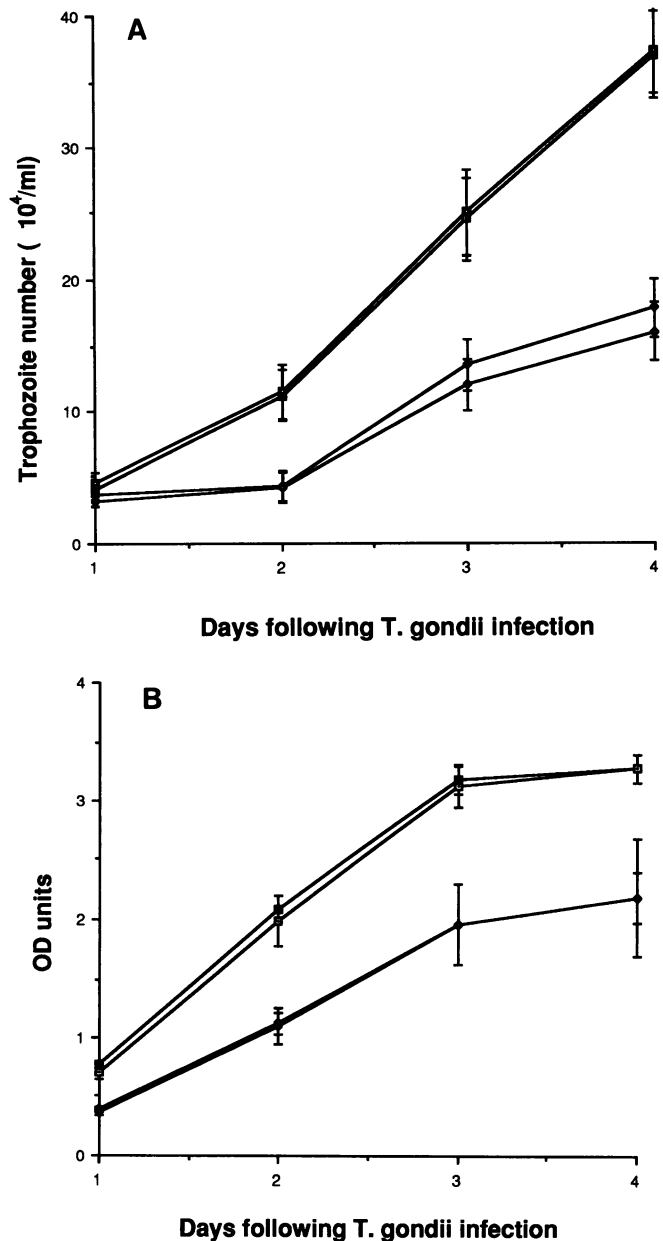


FIG. 1. *T. gondii* growth in HIV-infected or uninfected THP-1 cells. (A) Assessment of parasitic growth by trophozoite counting. Two *T. gondii*/cell ratios were used for infection. Three different experiments were performed, and each experiment was performed in quadruplicate. (B) Assessment of parasitic growth by measurement of p30 antigen by ELISA. Two *T. gondii*/cell ratios were used for infection. Three different experiments were performed, and each experiment was performed in 16 replicates. ◇, THP-1 cells infected with *T. gondii* in a ratio of one tachyzoite/two cells; ◆, THP-1 cells infected with HIV and *T. gondii* in a ratio of one tachyzoite/two cells; ■, THP-1 cells infected with HIV and *T. gondii* in a ratio of one tachyzoite/one cell. OD, optical density.

al. (13), obtained for macrophages coinfecting with HIV and *Mycobacterium avium*, *Leishmania chagasi*, or *Trypanozoma cruzi*, in which no reciprocal effect of infection was seen. It seems, therefore, that HIV-infected monocytes

TABLE 1. Effect of *T. gondii* infection on HIV replication in chronically HIV-infected THP-1 cells

Day after infection	RT activity (10^4 cpm/ 10^5 cells) ^a at given <i>T. gondii</i> /cell ratio		
	0	1:2	1:1
1	65 ± 11	92 ± 07	79 ± 03
2	72 ± 22	77 ± 07	62 ± 15
3	63 ± 13	66 ± 09	60 ± 06
4	64 ± 07	81 ± 12	72 ± 19

^a Data are presented as means ± SD for triplicate wells. Repeating the experiment three times gave similar results.

could be superinfected with other opportunistic pathogens with no apparent modulation of viral, parasitic, or bacterial replication. This is consistent with increasing evidence that HIV infection of macrophages produces only modest functional disturbances (10).

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