*Immunology* 1994 **83** 646–650

# Effect of IFN-γ on the proliferation of *Toxoplasma gondii* in monocytes and monocyte-derived macrophages from AIDS patients

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## SUMMARY

This study was undertaken to determine whether the activity of monocytes and monocyte-derived macrophages (MDM) from acquired immune deficiency syndrome (AIDS) patients against *Toxoplasma gondii* is altered and whether this activity can be modulated by recombinant interferon-γ (rIFN-γ). Untreated and rIFN-γ-treated monocytes or MDM from AIDS patients and from healthy controls were infected with *T. gondii* and the proliferation of these protozoa was determined. The $H_2O_2$ release by monocytes from AIDS patients and healthy controls was measured upon stimulation with phorbol myristate acetate (PMA) and formyl methionyl leucyl phenylalanine (FMLP). Monocytes from AIDS patients exhibited significantly lower toxoplasmastic activity compared to monocytes from healthy controls. The $H_2O_2$ release by monocytes from AIDS patients was also diminished. Incubation of monocytes from AIDS patients with rIFN-γ for 2 days, but not 1 day, restored their toxoplasmastatic activity. The rate of proliferation of *T. gondii* was higher in MDM from AIDS patients than in MDM from healthy controls. Treatment of MDM from AIDS patients with rIFN-γ for 1, 2 or 3 days resulted in partial inhibition of the proliferation of *T. gondii*. Collectively, these results demonstrate that the reduced toxoplasmastatic activity of monocytes and MDM from AIDS patients can be enhanced by *in vitro* treatment with rIFN-γ, which supports the clinical use of rIFN-γ for the treatment of opportunistic infections in these patients.

## INTRODUCTION

Patients with acquired immune deficiency syndrome (AIDS) frequently suffer from opportunistic infections with facultative or obligate intracellular micro-organisms, such as *Pneumocystis carinii*, *Candida albicans*, *Toxoplasma gondii* and *Mycobacteria*. Exudate macrophages, which are extravascular monocytes,[1] and activated macrophages are the most important effector cells against these pathogens.[2] Monocytes from patients with AIDS exhibit reduced clearance of opsonized erythrocytes by macrophages *in vivo*,[3] *in vitro* phagocytosis of opsonized *Staphylococcus aureus*,[4] intracellular killing of *S. aureus*[5] and *C. pseudotropicalis*,[6] generation of reactive oxidative radicals,[7] chemotactic activity,[8,9] induction of a T-lymphocyte proliferative response to various stimuli,[10–12] antibody-dependent cell-mediated cytotoxicity,[13] and expression of tissue factor by monocytes.[14] Some of these studies report that the functions of monocytes decrease when human immunodeficiency virus (HIV)-positive patients progress towards AIDS. Conflicting data have been published on the anti-microbial activity of monocytes and macrophages from HIV-infected individuals against the obligate intracellular pathogen *T. gondii*. The rate of proliferation of *T. gondii* was enhanced in interferon-γ (IFN-γ)-activated monocyte-derived macrophages (MDM) from HIV-positive individuals and patients with persistent generalized lymphadenopathy.[15] However, untreated and IFN-γ-treated monocytes, MDM and alveolar macrophages from patients with AIDS did not differ in toxoplasmastatic activity from cells from healthy controls.[15–17] The aim of the present study was to investigate whether the toxoplasmastatic activity of monocytes and MDM from AIDS patients is altered and whether this activity can be modulated by recombinant (r)IFN-γ.

Received 8 March 1994; revised 5 May 1994; accepted 10 August 1994.

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## MATERIALS AND METHODS

*Subjects*

The 43 AIDS patients under study belonged to group IV, according to the Centers for Disease Control and Prevention classification of 1987. The median $CD4^+$ lymphocyte count was 40/mm$^3$ (range 5–400/mm$^3$). Thirty-five patients received zidovudine as anti-retroviral drug, and 31 patients a low-dose cotrimoxazole to prevent *Pneumocytis carinii* penumonia. Healthy blood donors served as controls.

*Culture of monocytes and MDM*

Monocytes were isolated by Ficoll–amidotrizoate gradient centrifugation from blood containing sodium citrate as anticoagulant. In short, blood was diluted 1:2·5 with phosphate-buffered saline (PBS) and 25 ml of each suspension was layered over 10 ml of Ficoll–amidotrizoate ($\rho$ = 1·077). After centrifugation (440 *g* for 20 min at 18°) mononuclear cells were collected into conical tubes (Greiner, Frickenhausen, Germany) and washed twice with ice-cold PBS containing 0·5 U/ml heparin. Next the cells were counted and resuspended in Dulbecco's modified Eagle's medium containing 2 mM L-glutamin, 100 U/ml penicillin G and 0·1 mg/ml streptomycin (DMEM-plus), and 1% inactivated fetal calf serum (IFCS; Flow Laboratories, Irving, UK) at a concentration of $1 \times 10^6$ monocytes/ml. One millilitre of this suspension was pipetted onto plastic coverslips (Thermanox, Nunc Inc., Naperville, IL) in 24-well plates (Costar, Cambridge, MA). After 1 hr of adherence at 37° in 5% $CO_2$, non-adherent cells were washed off by vigorous rinsing of the coverslips in warm (37°) PBS. Coverslips contained more than 95% monocytes, as determined by morphological assessment. Monocytes were cultured for 7 days in order to differentiate into MDM in the presence of 20 ng/ml of granulocyte–macrophage colony-stimulating factor (GM-CSF; Sandoz, Vienna, Austria), which was added to DMEM-plus containing 10% IFCS at the start of the culture period. Monocytes and MDM were either used directly in the toxoplasma assay (see below) or cultured first for 1, 2 or 3 days in DMEM-plus containing 10% IFCS and various concentrations of rIFN-γ (a gift from Dr P. H. van der Meide, Department of Virology, TNO-ITRI, Rijswijk, the Netherlands), which was present only before infection with *T. gondii*.

*Proliferation of* T. gondii *in monocytes and MDM*

The toxoplasma assay was performed as described elsewhere, with minor modifications.[18] In short, coverslips with $1 \times 10^6$ monocytes or MDM were incubated in DMEM-plus containing 10% IFCS with toxoplasmas (RH strain) at a ratio of 1:1 for 30 min at 37° in 5% $CO_2$. After rinsing with warm (37°) PBS to remove non-ingested toxoplasmas, all the coverslips except one were incubated in DMEM-plus containing 10% IFCS for another 20 hr. After the coverslips were fixed for 20 min in methanol and stained with Giemsa stain, the number of toxoplasmas per cell at 0 hr and 20 hr was determined. The fold increase (FI) in the number of *T. gondii*, which is the number of toxoplasmas per positive cell at 20 hr divided by the number of toxoplasmas at 0 hr, was calculated.[18]

*Measurement of $H_2O_2$*

After isolation by Ficoll–amidotrizoate gradient centrifugation, $1 \times 10^6$ monocytes were placed in 3-ml plastic tubes (Greiner) under non-adherent conditions for measurement of $H_2O_2$ release. The $H_2O_2$ release by monocytes upon stimulation with 10 ng/ml phorbol myristate acetate (PMA; Sigma Chemical Co., St Louis, MO) and 500 nM formyl methionyl leucyl phenylalanine (FMLP; Sigma) was assayed by horseradish peroxidase-mediated $H_2O_2$-dependent oxidation of homovanillic acid[19] and expressed as nmol $H_2O_2/10^6$ monocytes/hr.

*Statistical analysis*

Each experiment included monocytes or MDM from patients and from healthy blood donors (controls). Statistical analysis of the data was performed with the Wilcoxon-signed rank test.

## RESULTS

### Toxoplasmastatic activity of monocytes and MDM and $H_2O_2$ release by monocytes from AIDS patients and controls

Monocytes from healthy controls which were not cultured before the toxoplasma assay inhibited the proliferation of *T. gondii*, which resulted in a low FI of about 3; MDM from healthy controls displayed much higher FI of toxoplasmas (about 6·5) (Fig. 1). The FI of toxoplasmas in monocytes and MDM from AIDS patients was significantly higher than in the respective cells from healthy controls (Fig. 1). The release of $H_2O_2$ by monocytes from AIDS patients upon stimulation with PMA and FMLP was reduced compared to control monocytes (Table 1).

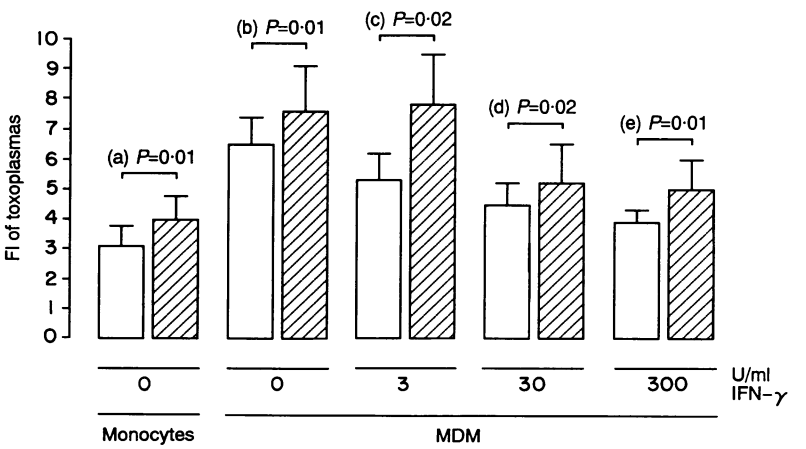


**Figure 1.** FI of *T. gondii* in freshly isolated monocytes and MDM from controls (□) and AIDS patients (▨) incubated with or without IFN-γ. Values represent mean ± SD; *n* = number of individuals. (a) *n* = 22; (b) *n* = 14; (c) *n* = 6; (d) *n* = 14; (e) *n* = 9.

**Table 1.** $H_2O_2$ release by monocytes from AIDS patients and controls*

| | | Stimulus | |
|---|---|---|---|
| | *n* | PMA | FMLP |
| Controls | 12 | 11·0 ± 2·1 | 4·4 ± 1·5 |
| AIDS patients | 12 | 7·1 ± 2·1† | 2·8 ± 1·6‡ |

*Results expressed as nmol of $H_2O_2$ released by $10^6$ cells/hr; mean values ± SD.
†$P = 0{\cdot}02$ compared to controls.
‡$P = 0{\cdot}03$ compared to controls.

### Toxoplasmastatic activity of monocytes and MDM from AIDS patients and controls treated with rIFN-γ

Incubation of control monocytes with 300 U/ml rIFN-γ for 1 day reduced the FI of *T. gondii*, but monocytes from only three out of seven AIDS patients responded to this treatment (Fig. 2). However, after 2 days of rIFN-γ treatment the impaired toxoplasmastatic activity of AIDS monocytes was restored (Fig. 2). MDM from healthy controls responded after 1 day of treatment with rIFN-γ, with a decrease in FI of toxoplasmas in a dose-dependent manner (Fig. 1). MDM from AIDS patients responded to a dose of 30 U/ml rIFN-γ, but the FI of toxoplasmas did not decrease further when these MDM were incubated with 300 U/ml rIFN-γ for 1 day (Fig. 1). Two or three days of treatment of these cells with 300 U/ml rIFN-γ did not augment the effect on the proliferation of *T. gondii* (Fig. 3).

### Effect of zidovudine on the toxoplasmastatic activity of monocytes

Treatment of patients with zidovudine leads to peak serum levels between 0·5 and 2·5 μg/ml.[20] To exclude the possibility that treatment of AIDS patients with zidovudine affected the toxoplasmastatic activity of monocytes, cells from healthy controls were incubated for 20 hr with zidovudine at doses of up to 10 μg/ml before performing the toxoplasma assay. The results showed that zidovudine had no effect on the toxoplasmastatic activity of monocytes (control FI = 4·3; 1 μg/ml zidovudine FI = 4·0; $n = 2$).

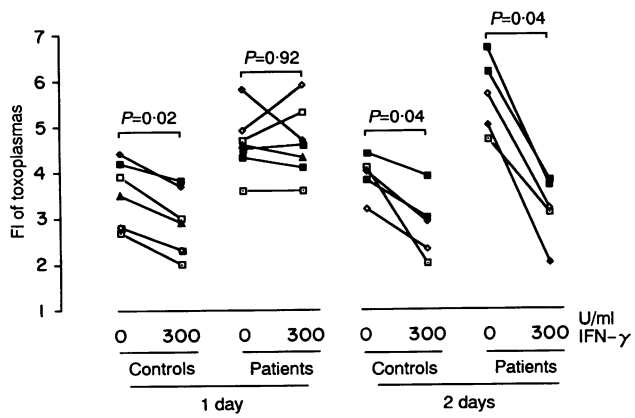


**Figure 2.** The effect of incubating monocytes from controls and AIDS patients with IFN-γ during 1 and 2 days on the FI of *T. gondii*.

## DISCUSSION

The present study demonstrates that the antimicrobial activity of monocytes and MDM from AIDS patients against *T. gondii* is reduced compared to that of cells from healthy donors. Treatment of monocytes with rIFN-γ for 2 days restored their toxoplasmastatic activity, whereas MDM only partially responded to 2 or 3 days of treatment with rINF-γ. The present findings are in agreement with the results of a previous study of the function of monocytes from AIDS patients in which *in vivo* treatment with rIFN-γ restored the toxoplasmastatic activity of the monocytes.[21] The *in vitro* incubation of monocytes with rIFN-γ for 2 days may mimic the condition occurring in patients that receive a daily injection of rIFN-γ, since monocytes reside for 2–3 days in the circulation.[22]

In other studies, the toxoplasmastatic activity of monocytes and non-activated or IFN-γ-activated MDM and alveolar macrophages from AIDS patients did not differ from that of cells from healthy controls.[16,17] MDM and alveolar macrophages from AIDS patients were activated by continuous incubation with IFN-γ for 3 days.[16] In our study, MDM already responded after 1 day of treatment with various concentrations of IFN-γ. Others have reported reduced activity against *T. gondii* of MDM from HIV-infected individuals, but not AIDS patients, after incubation of the cells with IFN-γ for 1 day.[15] It is not clear why our findings and those of other studies differ; clinical differences between the AIDS patients under study might play a role. The majority of AIDS patients studied by others[15,16] suffered from Kaposi's sarcoma (KS). This applied to only seven of the 43 patients in our study; moreover no differences in the FI of toxoplasmas in monocytes and MDM from AIDS patients with or without KS were observed (data not shown).

A possible effect of either the medication of AIDS patients or GM-CSF in the cell culture on the toxoplasma assay has been considered. The majority of the AIDS patients included in this study received cotrimoxazole, which could affect the proliferation of *T. gondii* in monocytes. However, no differences in the FI in monocytes were observed between AIDS patients with and without this medication (data not shown). An effect of zidovudine on the toxoplasmastatic activity of monocytes was excluded by performing the toxoplasma assay with monocytes from healthy individuals in the presence of zidovudine at concentrations which generally occur in the blood after treatment. Control experiments showed that addition of GM-CSF at the start of the 7-day culture of monocytes did not have an effect on the proliferation of *T. gondii* in MDM not treated with rIFN-γ or MDM incubated with rIFN-γ 1 day before the infection with *T. gondii* at the end of the culture period (data not shown).

The reduced toxoplasmastatic activity of AIDS monocytes is likely to be caused by a reduced release of reactive oxygen intermediates (ROI), such as $H_2O_2$, as has been demonstrated in this and other studies.[7,21] ROI are essential for inhibition of the proliferation of *T. gondii* in human monocytes and macrophages.[23] The low production of $H_2O_2$ by monocytes from AIDS patients may be attributable to the relatively poor cytokine environment, due to the reduced number of T lymphocytes,[24] resulting in non-optimal activation of

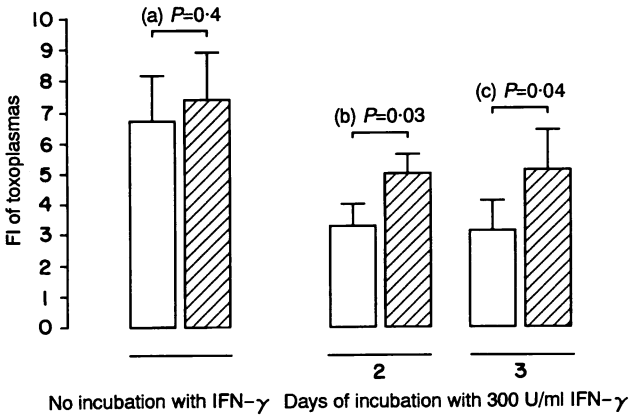


**Figure 3.** The effect of incubating MDM from controls (□) and AIDS patients (▨) with 300 U/ml IFN-γ during 2 and 3 days on the FI of *T. gondii*. Values represent mean ± SD. *n* = number of individuals. (a) *n* = 8; (b) *n* = 6; (c) *n* = 5.

monocytes *in vivo*. However, if this were the case, one would expect a response of monocytes from AIDS patients after 1 day of treatment with rIFN-γ, which was not observed. Another explanation for the reduced release of $H_2O_2$ by monocytes from AIDS patients is HIV infection of these cells. *In vitro* infection of monocytes with HIV resulted in a reduced release of ROI.[25] Only a very small percentage (0·001%) of mononuclear cells (monocytes and lymphocytes) from AIDS patients is infected with HIV.[26] A recent study, however, demonstrated that a substantial percentage (1·6–4·6%) of peripheral blood mononuclear cells from AIDS patients (stage IV) contains HIV provirus.[27] Therefore, it remains uncertain whether HIV can affect directly monocyte functions *in vivo*. Reduced antimicrobial activity can also be induced through interaction between monocytes and retroviral proteins. HIV glycoprotein 120 inhibited the chemotactic response upon stimulation with C5a and FMLP because of down-regulation of the receptors for these chemotactic factors.[28] Such a modulation of the FMLP receptors on monocytes from AIDS patients could explain the reduced release of $H_2O_2$ upon stimulation with this ligand. Furthermore, retroviral proteins can interfere with the signal transduction pathways involved in the antimicrobial activity of monocytes (P. H. Nibbering, personal communication), as reported for other types of cell.[29]

The clinical relevance of our findings is that up-regulation of the toxoplasmastatic activity of monocytes and MDM from AIDS patients by *in vitro* treatment with rIFN-γ supports the policy of adjunctive therapy with this cytokine for AIDS patients with an infection that does not respond adequately to antimicrobial therapy.

## ACKNOWLEDGMENTS

This study was supported by a grant for AIDS research from the Ministry of Welfare, Health and Culture of the Netherlands (grant number 88-89/89032).

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