# Interferon-gamma: A potent antiviral agent targeting macrophages infected with LP-BM5 murine leukemia virus, the causative agent of 'AIDS' in mice

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**JJ KORT, JL EISEMAN. Interferon-gamma: A potent antiviral agent targeting macrophages infected with LP-BM5 murine leukemia virus, the causative agent of 'AIDS' in mice. Can J Infect Dis 1992:3(Suppl B):115B-122B.** Cells of the monocyte/macrophage lineage (MM cells) are known to be infected by retroviruses, including the human immunodeficiency virus (HIV), without cytopathic changes and may serve as a persistent reservoir for the virus during the development of immunodeficiency disease. LP-BM5 murine leukemia virus (MuLV) infection of C57BL/6 mice and cell lines has been used to optimize therapy directed against macrophages. Findings in this murine system may be applicable to HIV infection in humans. The effect of recombinant murine interferon-gamma (IFN-γ) and 3′-azido-2′,3′-dideoxythymidine (AZT) as single agents or in combination was investigated in both LP-BM5 MuLV de novo infection and chronic infection of macrophages. Results indicate that the therapeutic effects of these single agents were dose-dependent and both agents were similarly effective in reducing the production of infectious virus determined by XC-plaque assay and by measurements of reverse transcriptase activity in culture supernatants; and AZT and IFN-γ reduced the production of virus proteins, quantified by laser densitometry of fluorographs from immunoprecipitated viral proteins using virus-specific antiserum. A combination of IFN-γ and that AZT showed greater antiviral activity in both LP-BM5 MuLV de novo and chronic infection of macrophages than either agent alone, suggesting that IFN-γ and AZT represent a potent combination of antiviral agents targeting macrophages. Further, since a lower concentration of each agent was required for efficacy in combination therapy, toxicity associated with single agent therapy may be avoided.

**Key Words:** *Interferon-gamma, LP-BM5 MuLV, Macrophages, Murine acquired immune deficiency syndrome*

## Interféron-gamma: agent anti-viral puissant, visant les macrophages infectés par le virus LP-BM5 de la leucémie murine, cause du «SIDA» chez les souris

Les cellules de lignées monocytes/macrophages (MM cells) sont infectées par des rétrovirus, y compris le virus de l'immunodéficience humaine (VIH), sans modifications cytopathiques et peuvent servir de réservoir permanent pour le virus au cours du développement de la maladie immunitaire. L'infection au virus de la leucémie murine (MuLV) LP-BM5 chez 6 souris/C57BL et des lignées cellulaires ont été utilisées dans le but d'optimiser le traitement dirigé contre les macrophages. Les résultats dans ce système murin peuvent être applicables à l'infection au VIH chez les humains. L'effet de l'interféron-gamma murin recombinant (IFN-γ) et du 3′-azido-2′,3′-didéozythymidine (AZT) à titre d'agents utilisés seuls ou en association, a été

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étudié dans l'infection de novo au MuLV LP-B5 et dans l'infection chronique des macrophages. Les résultats indiquent que les effets thérapeutiques de ces agents utilisés seuls sont dose-dépendants et que les deux agents se sont révélés aussi efficaces à réduire la production du virus infectieux, déterminé par un dosage sur plaque-XC et par des mesures de l'activité de la transcriptase inverse dans des surnageants de cultures. L'AZT et l'IFN-γ ont réduit la production de protéines virales, comme démontré par une densitométrie au laser d'une fluorographie effectuée sur des protéines virales immuno-précipitées, grâce à un antisérum spécifique. Une combinaison de l'IFN-γ et de l'AZT a montré une activité anti-virale supérieure, tant dans les cas d'infection nouvelle au MuLV LP-BM5 et d'infection chronique des macrophages, à celle de l'un ou l'autre des agents utilisé seul, ce qui donne à penser que l'IFN-γ et l'AZT représentent une association puissante d'agents antiviraux dirigés contre les macrophages. De plus, puisqu'une concentration plus faible de chacun des agents est nécessaire pour obtenir un traitement d'association efficace, la toxicité liée à chacun des médicaments est ainsi évitée.

IN THE PAST FEW YEARS, EVIDENCE HAS ACCUMULATED THAT cells of the monocyte/macrophage lineage (MM cells) function as a persistent reservoir for retroviruses and may be involved in the progression of retrovirus-induced acquired immune deficiency syndromes (AIDS) in humans and mice (MAIDS) by controlling production and dissemination of the virus (1-4). Extensive research has identified MM cells as central modulators of the immune system due to their unique repertoire of functions as: antigen-presenting cells; producers of cytokines and other factors (interleukin-1 [IL-1], IL-6, tumour necrosis factor-alpha, interferon-alpha [IFN-α], granulocyte colony stimulating factor [G-CSF], macrophage colony stimulating factor, prostaglandins, etc); phagocytes; and tumoricidal, cytotoxic cells (5-10).

The idea that alterations of MM cell functions occur after retrovirus infection and account in part for immune system suppression is controversial (11-16) and needs further investigation. However, development of a therapy directed against retrovirus infection of macrophages may limit dissemination and production of the retrovirus and control the progression of the disease (ie, AIDS and MAIDS). The authors have focused their efforts on the development of a treatment regimen against retrovirus infection of macrophages by using LP-BM5 murine leukemia viruses (MuLVs) in cultured murine macrophages. LP-BM5 MuLV is a mixture of replication-competent ecotropic and mink cell focus-inducing (MCF) MuLVs, and replication-deficient BM5 MuLV (which causes a lymphoproliferative/immunosuppressive syndrome in mice [MAIDS] – similar to AIDS in humans) (17-19).

Effects of recombinant murine IFN-γ and 3′-azido-2′,3′-dideoxythymidine (AZT) as single agents or in combination were tested in both de novo and chronic infections of macrophages with LP-BM5 MuLV. Single agents such as AZT and other dideoxynucleosides (dideoxycytosine [DDC], -adenosine [DDA] and -inosine [DDI]) inhibit human immunodeficiency virus (HIV) replication in lymphocytes (20,21) to a greater extent than in MM cells (22); this has been correlated to a diminished activity of dideoxynucleoside kinases in human MM cells which phosphorylate AZT and other dideoxynucleosides into active metabolites (22,23). Doses of these agents which would partially overcome the block of phosphorylation cause severe hematopoietic toxicity (AZT), neurotoxicity (DDC) and nephrotoxicity (DDA) (24-27). IFN-γ has been recognized as a potent inducer of a variety of macrophage functions leading to an 'antiviral state of the cell' (28-30), and it has been proposed that the decrease of IFN-γ-producing T cells in AIDS leads to an impaired activation of macrophages (31). Therefore the authors initiated investigations using IFN-γ as a therapy against retrovirus infection of macrophages (32,33).

## METHODS

**Cells and viruses:** The C-III macrophage cell line used in this study was originally isolated from the adherent population of cells derived from bone marrow of a C57BL/6 mouse. The C-III macrophages were cloned by a series of limiting dilutions in the presence of recombinant human macrophage CSF (Genzyme Corp, Massachusetts). More than 80% of the cells stained positive for nonspecific esterase by histochemistry and more than 90% expressed MAC-1 antigen on the cell surface as determined by fluorescence activated cell sorter (FACS) analysis. Electron microscopy of C-III cells is consistent with typical morphology of macrophages. Uninfected and LP-BM5 MuLV-infected macrophages were cultured in Dulbecco's modified eagle medium (DMEM) (Gibco Lab Inc, New York), supplemented with 5% fetal calf serum (FCS) (Hyclone Lab Inc, Utah) and antibiotics (5000 U/mL penicillin, 5 μg/mL streptomycin) at 37°C and 5% carbon dioxide in air atmosphere.

The mixture of LP-BM5 MuLV containing replication-competent ecotropic MuLV, MCF-MULV and replication-deficient BM5 MuLV was propagated in SC-1 cells, a fibroblastoid cell line susceptible to LP-BM5 MuLV infection. LP-BM5 MuLV containing culture supernatant from infected SC-1 cells was used to infect macrophage C-III cells. LP-BM5 MuLV-infected macrophage C-III cells produce infectious virus particles as determined by XC-plaque assay, measurements of the activity of reverse transcriptase (RT) in supernatants of infected macrophages and electron microscopy. In addition, both LP-BM5 MuLV-infected macrophage C-III cells and culture supernatant caused MAIDS in 100% of intraperitoneally inoculated mice.

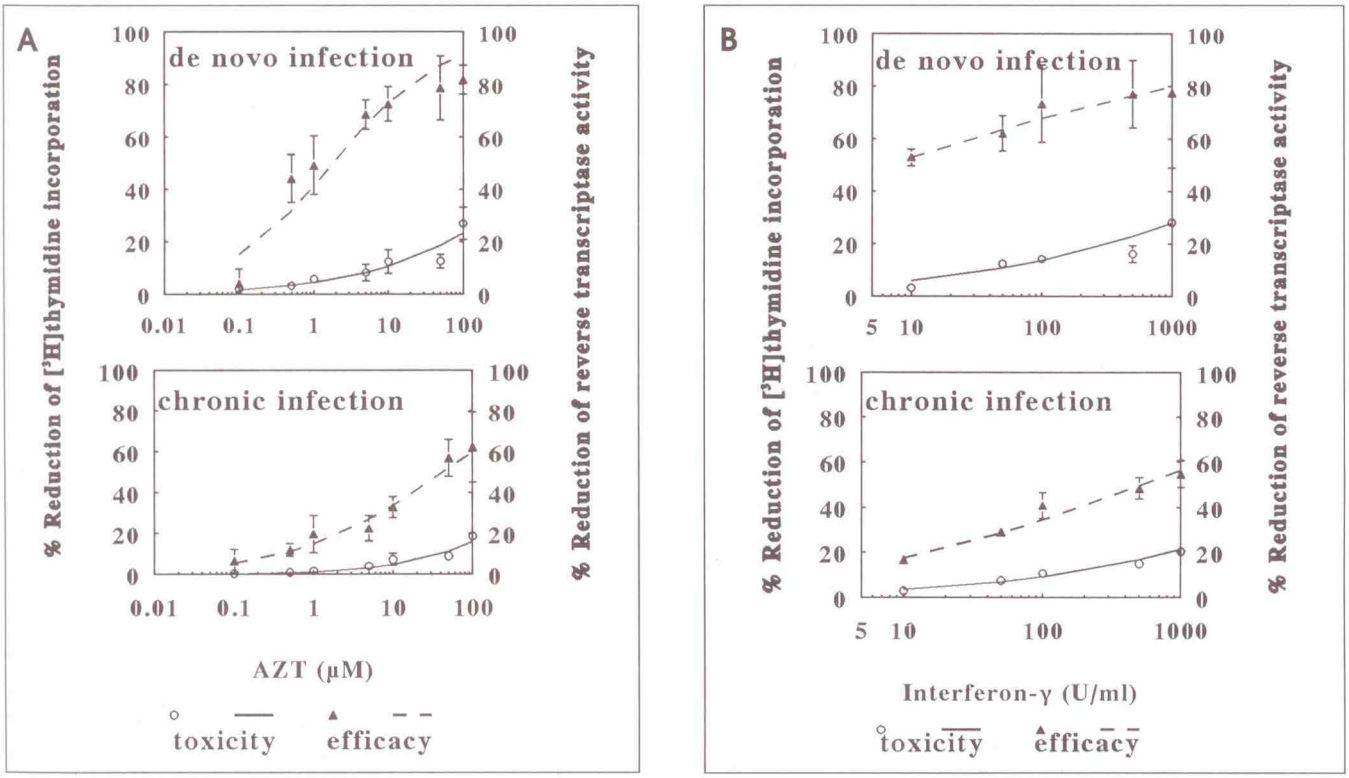



**Figure 1)** *Toxicity and antiviral activity of 3'-azido-2',3'-dideoxythymidine (AZT) and interferon-gamma (IFN-γ) in LP-BM5 murine leukemia virus (MuLV) de novo infected and chronically infected cultured murine macrophages. Toxicity was determined by [³H]-thymidine incorporation and antiviral activity (efficacy) by reverse transcriptase assay for AZT* **(A)** *and IFN-γ* **(B)**. *Each symbol represents mean ± SD of duplicates from three separate determinations. Curves were obtained by fitting the data to the Hill equation using Adapt software (37)*

**XC-plaque assay:** Detection of ecotropic MuLV was essentially performed according to the XC-plaque technique described by Rowe and colleagues (34) – carried out with $10^4$ cells per well in six-well plates. Syncytia formation was quantified by phase contrast microscopy and using a video camera supported colony counter system (Artek counter 982, Dynatech Lab, Virginia). Both methods gave similar counts of syncytia.

**Reverse transcriptase assay and [$^3$H]-thymidine incorporation:** The activity of RT in supernatants of LP-BM5 MuLV de novo and chronically infected macrophages was determined via a microtitre assay, modified from the procedures described by Gregersen et al (35) and Somogyi and co-workers (36). Uninfected macrophages and LP-BM5 MuLV chronically infected macrophages ($2.5 \times 10^3$ cells per well) were seeded in a volume of 100 μL per well of DMEM plus 5% FCS and 4 μg/mL polybrene into 96-well, flat bottom tissue culture plates (Nunc, Illinois) and were allowed to adhere. Different concentrations of IFN-γ (0, 10, 50, 100, 500 or 1000 U/mL) and AZT (0, 0.1, 0.5, 1, 5, 10 or 100 μM) or a combination (10 or 100 U/mL IFN-γ with 0.1, 0.5, 1 or 5 mM AZT) were added to each well in 50 mL aliquots 4 h prior to the addition of 50 μL/well of LP-BM5 MuLV containing clarified culture supernatant from infected SC-1 cells to uninfected macrophages and 50 μL per well or supernatant from uninfected SC-1 cells to LP-BM5 MuLV chronically infected macrophages. Cells were incubated for 24 h, washed twice with phosphate buffered saline and fed with DMEM (containing 5% FCS and the aforementioned concentrations of IFN-γ and AZT). Macrophage cultures were grown to confluency and 50 μL of supernatant were removed from each well for detection of RT. One microcurie per well of [$^3$H]-thymidine was added to a volume of 50 μL fresh culture medium to the remaining macrophage cultures, and cells were pulsed for 4 h in an incubator. Cells were lysed with deionized water and the released DNA was collected on glass fibre filters (Cambridge Technology Inc, Massachusetts) by using a PHD cell harvester (Cambridge Technology Inc). Filters were washed four times with deionized water and dried with methanol. [$^3$H]-thymidine incorporated into nascent DNA was quantified by liquid scintillation counting (LS 5801, Beckman Instruments Inc, California). For determination of RT activity, previously collected culture supernatants were clarified by low speed centrifugation (2000 *g* x2 mins) and added to a microtitre plate to a reaction mixture on ice containing 50 mM Tris-hydrochloride (pH 7.4), 5 mM dithiotreitol, 150 mM potassium chloride, 5 mM manganese chloride, 1.5 mM glutathione, 1 mM EGTA, 40 μg/mL template primer (poly (rA):p(dT)$_{12\text{-}18}$, Pharmacia, New Jersey) and 0.5% Triton X-100 in a total volume of 100 μL aqueous solution (final

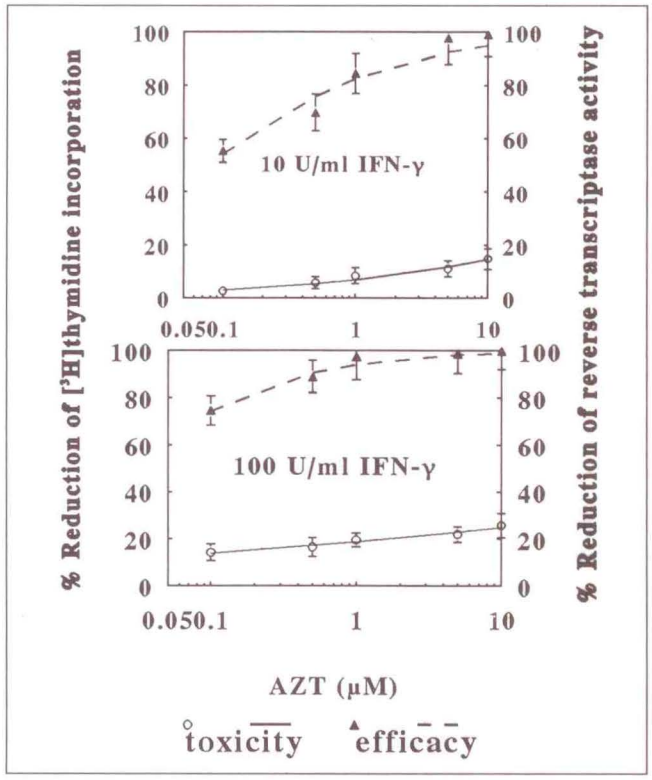



**Figure 2)** *Toxicity and antiviral activity of a combination of 3'-azido-2',3'-dideoxythymidine (AZT) with interferon-gamma (IFN-γ) in LP-BM5 murine leukemia virus (MuLV) de novo infection of cultured murine macrophages. Toxicity was determined by [³H]-thymidine incorporation and antiviral activity (efficacy) by reverse transcriptase assay. Each point represents mean ± SD of duplicates from three separate experiments. Curves were calculated by fitting the data to the Hill equation using Adapt software (37)*

concentrations). The mixture was placed on ice for 30 mins before the reaction was started by adding 4 μCi per well [$^3$H]deoxythymidine-5'-triphosphate (DuPont, Massachusetts). Microtitre plates were sealed with parafilm and incubated for 2 h at 37°C in a water bath with agitation every 15 mins. The reaction was stopped by transferring the plates to ice. Two DE81 ion exchange filter paper disks (Whatman Inc, New Jersey) were placed into each well of the microtitre plates and the plates were incubated for 1 h at 22°C. The DE81 filter disks were placed on a Bio-Dot apparatus (Bio-Rad, California) over 3 mm Whatman chromatography paper and washed five times with ice-cold 5% sodium hydrogen phosphate, twice with deionized water and twice with methanol. [$^3$H]-deoxythymidine monophosphate incorporated into nascent DNA was quantified by liquid scintillation counting of the DE81 paper disks. Results of the RT assay and [$^3$H]-thymidine incorporation are presented as percentage reduction compared with control (no treatment). Data were analyzed statistically by fitting them to the Hill equation, and concentrations for IFN-γ and AZT at which half maximal toxicity ($IC_{50}$) and half maximal antiviral activity ($EC_{50}$) would be expected were calculated by nonlinear reiteratively weighted regression analysis using Adapt software (37).

**[$^{14}$C]leucine incorporation into macrophages:** Macrophages were seeded out in microtitre plates, incubated with DMEM plus 5% (volume/volume) FCS containing 0, 1, 10, 50, 100, 500, 1000, 5000 or 10,000 U/mL IFN-γ. Cells were allowed to grow for two days and the culture medium, containing the different concentrations of IFN-γ, was changed every day. On the third day, cells were washed twice with leucine- and antibiotic-free modified DMEM and incubated for 4 h with leucine- and antibiotic-free, modified DMEM containing 5 μCi/mL [$^{14}$C]-leucine (DuPont) and the respective concentration of IFN-γ. Cells were lysed with distilled water and proteins were harvested on glass-fibre filters using a PHD cell harvester (Cambridge Technology Inc). Protein-incorporated [$^{14}$C]leucine was quantitated by liquid scintillation counting.

**Metabolic labelling of macrophages:** De novo and LP-BM5 MuLV chronically infected macrophages in six-well plates were grown for three days with or without the addition of IFN, AZT or a combination. The subconfluent monolayers of LP-BM5 MuLV de novo and chronically infected macrophages were washed twice with methionine-free modified DMEM and then incubated for 12 h with methionine-free modified DMEM containing 50 μCi/mL [$^{35}$S]methionine (DuPont), 5% FCS and the same addition of IFN-γ and AZT as before. The supernatant was removed and cell monolayers were washed twice with HEPES buffered saline (0.9% sodium chloride, 50 mM N-2-hydroxyethylpiperazine-N'-2-ethane-sulphonic acid, pH 7.4) prior to the addition of 0.5 mL per well ice-cold extraction buffer A containing 5 mM Tris-hydrochloride, 1 mM EDTA, 0.4 M potassium chloride, 1% Triton X-100, 1 mM phenylmethylsulphonylfluoride (Boehringer Mannheim, Indiana) and 1 mM tosylphenylchloroketone (Boehringer Mannheim). Cell extracts were used for immunoprecipitation.

**Immunoprecipitation of viral proteins:** Preparation of the cell extracts and immunoprecipitation with polyclonal, monospecific goat antisera to purified retrovirus protein $p30^{gag}$ was performed as described by Bilello et al (38). To compensate for differences in protein synthesis of the differently treated macrophages, each immunoprecipitation was done with approximately $2.5x10^7$ dpm of cell extract. The volume of each sample was adjusted to 1 mL with extraction buffer B (extraction buffer A without potassium chloride). Cell extracts were precipitated with normal goat serum prior to immunoprecipitation with virus-specific goat antiserum, containing αp30 antibodies. *Staphylococcus aureus* immunoabsorbant (Life Tech Inc, Maryland) was used to collect antigen-antibody complexes by centrifugation. Pellets were washed twice with a solution containing 20 mM Tris-hydrochloride (pH 7.4), 100 mM sodium chloride, 1 mM EDTA, and 0.5% Nonidet P-40 (Sigma Chemical Co, Missouri) and once with the same solution plus

2.5 mM potassium hydrochloride. Samples were prepared for sodium dodecylsulphate (SDS) polyacrylamide (12.5%) slab gel electrophoresis (PAGE) and SDS-PAGE was performed according to the method of Laemmli (39). Rainbow markers (Amersham Corp, Illinois) were used to estimate molecular weight of the proteins.

**Fluorography and quantification of labelled protein bands:** SDS-polyacrylamide slab gels were fixed for 30 mins with a solution of propanol-2:acetic acid:water (25:10:65) soaked in Amplify (Amersham Corp) for another 30 mins and dried at 70°C under vacuum. The dried gels were exposed to Chronex 4 x-ray films at -70°C. The amount of viral proteins present in the bands of fluorographs was quantified via an integrating laser densitometer (Ultroscan XL; Pharmacia LKB Biotechnology, New Jersey) and GelScanXL 2.1 software.

## RESULTS

The antiviral activity of AZT and IFN-γ as single agents and in combination was correlated to the reduction of RT activity in culture supernatants of LP-BM5 MuLV de novo infected and chronically infected macrophages. Both agents were similarly effective antivirals at concentrations where toxicity, measured as inhibition of [$^3$H]-thymidine incorporation, was low (Figure 1). IFN-γ and AZT were more effective antivirals against the de novo infection of macrophages than in chronic infection. Treatment of cells with combinations of IFN-γ (10 or 100 U/mL) and various concentrations of AZT resulted in greater reduction of RT activity than for either agent alone. In addition, no increased cytotoxicity was observed with combination regimens in the de novo infection of macrophages with LP-BM5 MuLV (Figure 2), suggesting that a combination of AZT and IFN-γ is more effective than either agent alone. Statistical analysis of the single-agent treatment regimen was carried out by nonlinear reiteratively weighted regression analysis (37) and concentrations of $EC_{50}$ and $IC_{50}$ were calculated for IFN-γ and AZT (Table 1).

Essentially the same results were obtained when the production of infectious virus was quantitated by XC-plaque technique in the presence of single agents (AZT, IFN-γ) or their combination (Table 2).

Little is known about the mechanism by which IFN-γ decreases the production of infectious virus in macrophages. The authors examined this mechanism by investigating the effect of IFN-γ on production of viral core proteins in LP-BM5 MuLV de novo and chronically infected macrophages by immunoprecipitation of [$^{35}$S]methionine metabolically labelled virus proteins with virus-specific antiserum (Figure 3). Quantitation of the 30 kD virus-specific bands ($p30^{gag}$) by laser densitometry indicated that: cell-derived virus protein $p30^{gag}$ was reduced by IFN-γ in a concentration-dependent fashion (Table 3) – the effect was greater in LP-BM5 MuLV de novo infected macrophages than in chronically infected macrophages; and the amounts of

**TABLE 1**
**Toxicity and antiviral activity of 3′-azido-2′,3′-dideoxythymidine (AZT) and interferon-gamma (IFN-γ) in macrophages**

| Agent | Half maximal toxicity ($IC_{50}$) | Half maximal antiviral activity ($EC_{50}$) | Therapeutic index ($IC_{50}/EC_{50}$) |
|---|---|---|---|
| AZT (μM) | | | |
| De novo infection | 1809 | 1.85 | 972 |
| Chronic infection | 1438 | 41.81 | 34 |
| Interferon (U/mL) | | | |
| De novo infection | 11,392 | 6.55 | 1739 |
| Chronic infection | 18,896 | 509 | 37 |

*Values are mean contentrations at which $IC_{50}$ and $EC_{50}$ were estimated using Adapt software (37)*

**TABLE 2**
**Reduction of syncytia formation by 3′-azido-2′,3′-dideoxythymidine (AZT) and interferon-gamma (IFN-γ) using XC-plaque technique in LP-BM5 MuLV de novo and chronically infected macrophages**

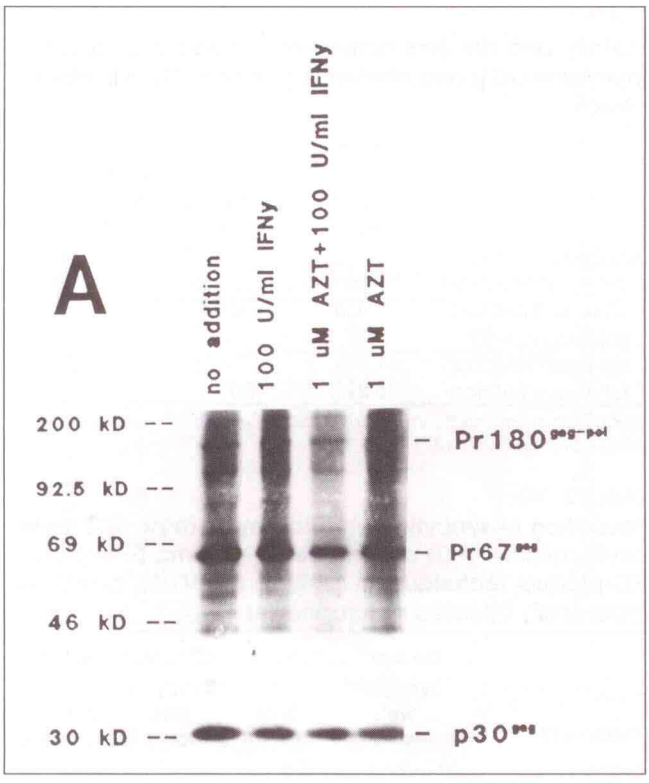
| Treatment | De novo infection Syncytia/well (mean ± SD) | % of control | Chronic infection Syncytia/well (mean ± SD) | % of control |
|---|---|---|---|---|
| None (control) | 49.0±22.1 | 100 | 195.6±32.6 | 100 |
| 1 U/mL IFNγ | 43.6±23.4 | 89.2 | ND | ND |
| 5 U/mL IFNγ | 41.0±20.9 | 83.7 | ND | ND |
| 10 U/mL IFNγ | 34.0±16.0 | 69.4 | 169.6±41.6 | 86.7 |
| 50 U/mL IFNγ | 13.3±7.5 | 27.2 | 83.0±25.1 | 42.4 |
| 100 U/mL IFNγ | 4.6±2.5 | 9.5 | 19.6±5.9 | 10.1 |
| 500 U/mL IFNγ | ND | ND | 5.6±3.1 | 2.9 |
| 1000 U/mL IFNγ | ND | ND | 2.3±1.2 | 1.4 |
| 1 μM AZT | 6.5±3.58 | 13.3 | 24.3±10.9 | 12.4 |
| 1 μM AZT + 100 U/mL IFN-γ | 0.3±0.6 | 0.7 | 2.6±1.2 | 1.4 |

*Values are from three independent experiments. ND Not determined*

**TABLE 3**
**Densitometric quantitation of viral core protein ($p30^{gag}$) production from LP-BM5 MuLV-infected macrophges using immunoprecipitation techniques**

| Treatment | De novo infection % of control | Chronic infection % of control |
|---|---|---|
| None (control) | 100 | 100 |
| 1 U/mL IFNγ | 89.7 | ND |
| 5 U/mL IFNγ | 79.2 | ND |
| 10 U/mL IFNγ | 60.9 | 99 |
| 50 U/mL IFNγ | 52.8 | 83 |
| 100 U/mL IFNγ | 25.0 | 74.3 |
| 500 U/mL IFNγ | ND | 65.2 |
| 1000 U/mL IFNγ | ND | 51.7 |
| 1 μM AZT | 71.3 | 77.6 |
| 1 μM AZT + 100 U/mL IFN-γ | 17.6 | 39.7 |

*Bands on fluorographs of immunoprecipitated viral proteins were scanned with an integrating laser densitometer and the area under the curve (AUC) of each $p30^{gag}$ band was calculated using GelScanXL 2.1 software (Pharmacia). Results are percentage of AUC of untreated controls. ND Not determined*



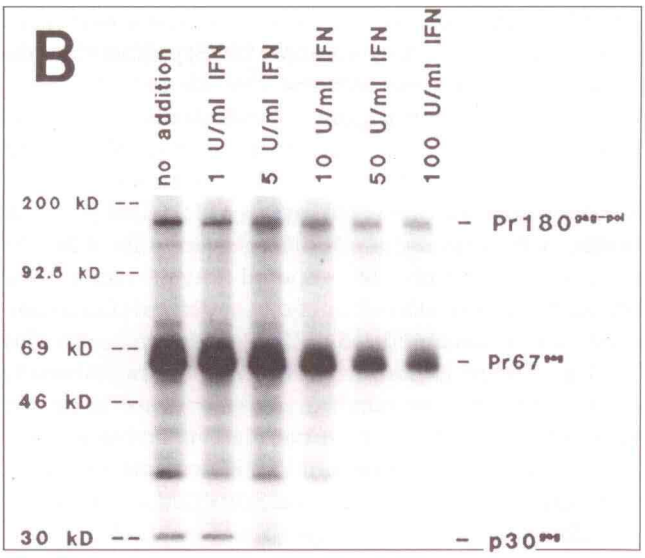




**Figure 3)** *Immunoprecipitation of [$^{35}$S]methionine-labelled viral proteins from LP-BM5 murine leukemia virus (MuLV) de novo and chronically infected macrophages with goat antiserum against p30$^{gag}$.* **A (left)** *LP-BM5 MuLV chronic infection.* **B (above)** *de novo infection with LP-BM5 MuLV. Prominent bands of the two major polyprotein precursors Pr180$^{gag-pol}$ and Pr67$^{gag}$, and the viral p30$^{gag}$ protein are indicated. Other bands represent breakdown products of the viral polyprotein precursors. AZT 3'-azido-2',3'-dideoxythymidine; IFN-γ Interferon gamma*

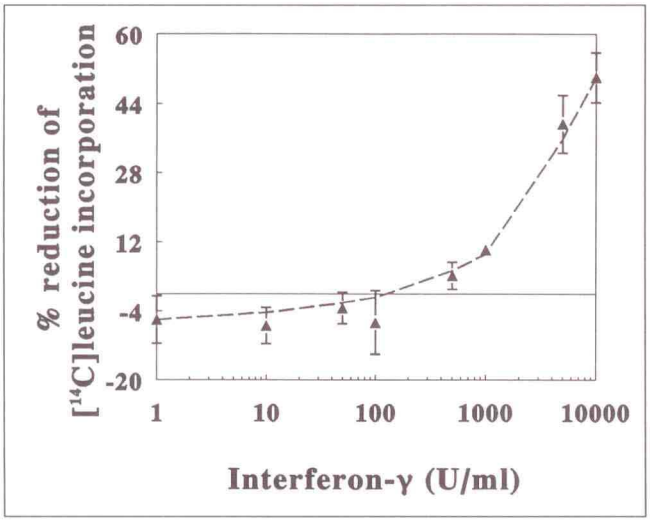



**Figure 4)** *Effect of interferon-gamma on steady-state protein synthesis in macrophages. Protein synthesis was correlated to [$^{14}$C]leucine incorporation. Data are expressed as percentage reduction of [$^{14}$C]leucine incorporation compared with untreated cells (ordinate). Negative values indicate increased [$^{14}$C]leucine incorporation compared with untreated cells. Symbols represent mean ± SD of duplicates from three separate experiments*

polyprotein precursors Pr67$^{gag}$ and Pr180$^{gag-pol}$ were also decreased by IFN-γ treatment, indicating that post translational cleavage of virus proteins was not significantly changed by IFN-γ. In addition, the combination of 100 U/mL IFN-γ and 1 μM AZT further reduced cell-associated virus protein (compared with the single agents), which parallels results of XC-plaque assays and measurements of the RT activity in supernatants of cultured cells presented above. However, the reduction in synthesis of viral proteins in LP-BM5 MuLV de novo and chronically infected macrophages by IFN-γ was of lower magnitude than the decreases seen in RT activity or in XC-plaques. De novo protein synthesis measured as [$^{14}$C]leucine incorporation in C-III macrophages was slightly stimulated by IFN-γ at concentrations below 100 U/mL (Figure 4) and, thus, it is likely that IFN-γ inhibits specifically the translation of virus proteins.

## DISCUSSION

Biological response modifiers (BRMs) are effective in the treatment of cancer, immunocompromized individuals and patients with hematological disorders. Most BRMs act locally at very low concentrations and are selective for specific cells in a defined microenvironment. Therapeutic approaches using systemic application of BRMs often are followed by toxic side effects due to the overall activation of target cells. IFN-γ has been recognized as the major product from T lymphocytes and is known to induce a variety of processes in macrophages leading to functional activation of the cells (30-32). With regard to the effects of IFN-γ on macrophages, the authors addressed the following questions: can macrophages be protected from retroviral infection? and does IFN-γ have an effect on virus expression in chronically infected cells using the LP-BM5 MuLV model? Results indicate that IFN-γ is more effective in reducing production of infectious virus from de novo infected macrophages than from chronically infected macrophages, an observation supported by the reduc-

tion of RT activity in supernatants of LP-BM5 MuLV-infected macrophages, by decreased formation of syncytia in an XC-plaque assay and by a diminished expression of viral core protein $p30^{gag}$ (and envelope protein $gp70^{env}$, unpublished data) by IFN-γ. Quantitative differences exist between the effects of IFN-γ on the production of infectious virus and expression of virus protein. The authors speculate that IFN-γ interferes with assembly and release of the virus, and leads to production of noninfectious virus particles in macrophages and 'trapping of virions' on the cell membrane, similar to using IFN-β in MCF-infected mouse fibroblasts (38,40). Since there is a reduction of virus polyprotein precursors ($Pr180^{gag\text{-}pol}$, $Pr67^{gag}$), IFN-γ alters translation of viral mRNA and may effect the level of transcription. The inhibitory effect of IFN-γ on production of virus protein can not be explained by inhibition of protein synthesis because incorporation of [$^{14}$C]leucine into proteins was slightly stimulated in macrophages at concentrations under 100 U/mL and was only moderately inhibited at higher concentrations of IFN-γ.

In conclusion, the present results indicate that IFN-γ is a potent BRM with antiviral activity against retrovirus infection of murine macrophages. These findings are in agreement with the recently reported protective effect of IFN-γ against HIV infection of macrophages (33). The enhanced inhibitory effect of IFN-γ and AZT on both LP-BM5 MuLV de novo and chronic infection of macrophages suggests that this combination warrants further testing in vitro and in vivo using combinations of IFN-γ and AZT with concentrations below the $EC_{50}$ of each agent to elucidate further the interaction between these two antiviral agents.

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