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## Notes

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# Tumor Necrosis Factor-α and Expression of the Multidrug Resistance-Associated Genes LRP and MRP

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***Background and Purpose:*** **Cancer cells that express P-glycoprotein, multidrug resistance-associated protein (MRP), or lung resistance protein (LRP) have demonstrated resistance to a wide variety of chemotherapeutic drugs. Recently, we reported that human colon carcinoma cells that express all three proteins exhibit reduced P-glycoprotein gene expression and a loss of multidrug resistance after exposure to tumor necrosis factor-α, a hormone-like protein produced by cells of the immune system. In this study, we examined the effects of tumor necrosis factor-α on MRP and LRP gene expression in the same colon carcinoma cells.** ***Methods:*** **HCT15 and HCT116 colon carcinoma cells were incubated with tumor necrosis factor-α at 100 U/mL for 2, 12, 24, 48, or 72 hours; alternatively, cells transfected with an expression vector containing a human tumor necrosis factor-α complementary DNA were studied. The effects of tumor necrosis factor-α on MRP and LRP messenger RNA expression were evaluated by means of reverse transcription and the polymerase chain reaction; effects on MRP and LRP protein expression were examined by use of specific monoclonal antibodies and flow cytometry. The flow cytometry data were analyzed by use of the two-sided, nonparametric Mann–Whitney rank sum test.** ***Results:*** **Treatment with exogenous tumor necrosis factor-α reduced the level of LRP messenger RNA in both cell types in an apparently time-dependent fashion; in HCT15 cells, almost no LRP messenger RNA was detected after 48 hours of treatment. In contrast, the level of MRP messenger RNA was increased in HCT116 cells by such treatment, but the level in HCT15 cells was unchanged. Treatment with exogenous tumor necrosis factor-α induced changes in LRP and MRP protein expression in the two cell types that paralleled the changes found for messenger RNA. In transfected cells, the endogenous production of tumor necrosis factor-α reduced LRP gene expression (both messenger RNA and protein) and increased MRP gene expression (both messenger RNA and protein), regardless of cell type.** ***Conclusion:*** **In human colon carcinoma cells, tumor necrosis factor-α influences MRP and LRP gene expression in opposite ways. The findings for LRP gene expression parallel our earlier findings for P-glycoprotein expression in these cells.** ***Implication:*** **In developing strategies for overcoming multidrug resistance in tumor cells, the possibility that an agent can suppress one or more mechanisms of drug resistance and enhance others should be considered. [J Natl Cancer Inst 1997;89:807-13]**

Numerous investigators (*1-8*) have reported that cytokines are able to potentiate drug activity in multidrug-resistant cell populations. These reports have suggested that the inclusion of cytokine administration in combination-chemotherapy protocols may provide a clinical approach to reversing multidrug resistance. This approach, however, is complicated by the toxic effects associated with systemic administration of cytokines such as tumor necrosis factor-α (TNF-α) (*9,10*). One possible method of administering TNF-α that may avoid systemic toxic effects is the direct introduction into

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*See* ''Notes'' following ''References.''

tumor cells of expression vectors encoding the cytokine. We have recently demonstrated the feasibility of this approach in vitro by transfecting P-glycoprotein-expressing, multidrug-resistant human colon carcinoma cells with an expression vector that uses the cytomegalovirus promoter to drive the expression of TNF-α (*11*). While the in vitro feasibility of this concept has been established, there remain many practical issues that must be resolved before the implementation of a gene therapy approach such as this one.

An additional complication for multidrug resistance-reversal strategies, in general, is the existence of other, non-P-glycoprotein-mediated mechanisms of resistance that may or may not be amenable to a given reversal approach. Concurrent operation of two distinct resistance mechanisms has been observed in a single tumor cell population (*12-14*). Furthermore, in immunohistochemical studies (*15*) of a large panel of human tumor cell lines, we have observed the frequent occurrence of as many as three overlapping phenotypes of multidrug resistance. Therefore, we were interested in investigating the generality of the TNF-α effect with respect to the expression of other multidrug resistance-associated genes.

The MRP gene was discovered in a multidrug-resistant, P-glycoprotein-negative tumor cell line (*16*). Subsequent cloning and transformation studies (*17*) gave evidence that this gene could confer resistance to a wide spectrum of drugs. The 180-195-kd membrane glycoprotein encoded by MRP, known as the multidrug resistance-associated protein, is a member of the superfamily of adenosine triphosphate-binding proteins that extrude a wide variety of structurally and functionally unrelated compounds from cells (*18*). Although there have been a number of studies [e.g., *19-21*] that have investigated MRP gene expression in different tumor types, the clinical significance of their findings for the treatment and/or prognosis of human cancer remains to be established.

The lung resistance protein (LRP) was also initially identified in a P-glycoprotein-negative, multidrug-resistant (lung) tumor cell line (*22*). An examination of complementary DNA (cDNA) sequence homologies led to the identification of this 110-kd protein as the major cytoplasmic vault protein and the suggestion that cytoplasmic vaults are organelles involved in nuclear-cytoplasmic transport (*23,24*). Expression of LRP has been investigated in numerous human tumor cell lines (*22*) and in normal human tissues and solid tumors, revealing a broad distribution of LRP expression and elevated levels in particular organs and tumor types [e.g., in digestive tract epithelial cells and in colorectal carcinomas (*25*)]. Data supporting the clinical significance of LRP gene expression in predicting the response to chemotherapy have been reported for both solid (*26,27*) and hematopoietic (*28*) cancers.

In this study, we examined the effects of exogenous TNF-α and endogenously produced TNF-α on LRP and MRP gene expression in human colon carcinoma cells that also express P-glycoprotein. We have previously shown that TNF-α treatment suppresses P-glycoprotein expression by these cells and sensitizes them to drugs (*8,11*).

## Materials and Methods

### Overall Approach

We analyzed TNF-α-treated and TNF-α cDNA-transfected human colon carcinoma cells to evaluate the cytokine's potential for modulating the expression of the multidrug resistance-associated genes LRP and MRP in the context of reversal of the multidrug-resistant phenotype. The TNF-α-mediated effects on LRP and MRP expression were analyzed by determining messenger RNA (mRNA) levels and protein levels and evaluating these expression levels in relationship to the results of functional assays, i.e., determinations of fluorescent drug accumulation and in vitro chemosensitivity to the drugs doxorubicin and vincristine, as previously described (*8,11*).

### Cell Lines

The human colon carcinoma cell lines HCT15 (*29*) and HCT116 (*30*) were characterized previously with regard to their expression of the MDR1 (also known as PGY1; P-glycoprotein) (*31*), the LRP, and the MRP (*15*) genes. While both cell lines demonstrate multidrug resistance in vitro, HCT15 cells are approximately two times more resistant than HCT116 cells to seven multidrug resistance-associated drugs (*15,31*). The cells were cultured as described previously (*8,11*).

### TNF-α Treatment

To investigate the influence of externally applied TNF-α (Promega Corp., Madison, WI; 100 U/mL) on LRP and MRP gene expression, HCT15 and HCT116 cells were treated for 2, 12, 24, 48, or 72 hours (*8*). The TNF-α-treated cells were used either for RNA isolation or for the detection of LRP and MRP protein.

### Construction of the TNF-α Expression Vector

The murine leukemia virus-derived plasmid expression vector pM3neo was used to construct the plasmid pM3CMV-hTNF, which contains a human TNF-α cDNA whose expression is driven by the cytomegalovirus promoter, as previously described (*11*).

### Transfection of Tumor Cells

Human colon carcinoma cells were transfected with pM3CMV-hTNF by means of electroporation (*32*), as described previously (*11*). The selection of neomycin-resistant clones was carried out in 0.8 mg/mL Geneticin (G418; Life Technologies, Inc. [GIBCO BRL], Gaithersburg, MD).

### RNA Isolation and Reverse Transcription–Polymerase Chain Reaction (RT–PCR)

Total RNA was isolated from cells by use of a miniprep-RNA protocol (*33*), and RT–PCR was performed as described previously (*8,11*). For the PCR step, TNF-α-, LRP-, MRP-, and β-actin-specific primers were used to amplify a 702-base pair (bp) product for TNF-α (*34*), a 405-bp product for LRP (the upstream primer corresponded to LRP cDNA residues 136-159: 5′-CCC CCA TAC CAC TAT ATC CAT GTG-3′; the downstream primer corresponded to residues 521-542: 5′-TGG AAA AGC CAG TCA TCT CCT G-3; the sequence of the downstream primer was based on prototype sequence information and deviates slightly from the actual LRP sequence [i.e., G rather than C at positions 2 and 12 of this 22-mer]), a 291-bp product for MRP (*19*), and a 316-bp product for β-actin (*31*). Semiquantitation of separated PCR products was done by means of video densitometry, using the Image 1.44 program (provided by Wayne Rasband, National Institute of Mental Health, Bethesda, MD).

### Detection of LRP and MRP Protein Expression With Specific Monoclonal Antibodies and Immuno-flow Cytometry

The preparation of HCT15 and HCT116 cells for this analysis was performed as described previously (*8-11*). The cells were incubated for 60 minutes at 4 °C with the appropriate primary monoclonal antibodies. The 110-kd-LRP protein was detected with the mouse antibody LRP-56 [(*22*); diluted 1:50 with 2% bovine serum albumin (BSA) in phosphate-buffered saline (PBS)], and the 190-kd MRP protein was detected with the rat antibody MRPr1 [(*35*); diluted 1:200 with 2% BSA–PBS]. Cells incubated with mouse immunoglobulin $G_1$ (Becton Dickinson, San Jose, CA) served as negative controls. Fluorescein-conjugated secondary antibodies were used to detect primary antibody binding. For LRP-56 and the control antibody, a goat-anti-mouse antibody (TAGO Inc., Burlingham, CA) was used, and, for MRPr1, a rabbit-anti-rat-antibody (Sigma Chemical Co., St. Louis, MO) was used. Incubations with the secondary antibodies were for 60 minutes at 4 °C. A

FACScan flow cytometer (Becton Dickinson) was used to measure the fluorescence intensity of $1 \times 10^4$ cells per group. Quantitation of the data was performed using the LYSIS program (Becton Dickinson).

### Statistical Analysis

The nonparametric, two-sided Mann–Whitney rank sum test was used to analyze the flow cytometry data. Data from at least three independent experiments were included in the analysis. Statistical significance was established on the basis of 95% confidence intervals.

## Results

### LRP and MRP mRNA Expression in TNF-α-Treated Cells

To determine the effects of externally added TNF on LRP and MRP mRNA expression, HCT15 cells and HCT116 cells were incubated with 100 U/mL TNF-α for 2, 12, 24, 48, or 72 hours. LRP, MRP, and β-actin mRNA levels were then examined by means of RT–PCR. LRP mRNA expression was reduced in TNF-α-treated cells of both lines compared with untreated cells, and the reductions were dependent on the duration of TNF-α exposure (Fig. 1, A and B). In HCT15 cells, almost no LRP mRNA was detectable after 48 and 72 hours of TNF-α treatment (Fig. 1, A). This time-related modulation of LRP gene expression was monitored in relation to the expression of β-actin mRNA, which encodes a housekeeping protein and whose expression is not influenced by TNF-α (*36*,*37*).

In contrast, MRP mRNA expression was increased in HCT116 cells after the external addition of TNF-α, seemingly in a time-related fashion. The highest mRNA levels for MRP were detected in these cells after 48 and 72 hours of TNF-α treatment (Fig. 1, D). In HCT15 cells, the colon carcinoma cells with the higher degree of multidrug resistance, the mRNA levels for MRP remained unchanged after incubation with TNF-α (Fig. 1, C).

### LRP and MRP Protein Expression in TNF-α-Treated Cells

To analyze the influence of externally applied TNF on LRP and MRP protein expression levels, HCT15 cells and HCT116 cells were again incubated with TNF-α for 2, 12, 24, 48, or 72 hours. The TNF-α-mediated modulation of protein expression was investigated with monoclonal primary antibodies (LRP-56 for LRP and MRPr1 for MRP), fluorescein-conjugated secondary antibodies, and flow cytometry as described in the ''Materials and Methods'' section. In comparison with nontreated cells, which had mean LRP fluorescence/cell values of 122 for HCT15 cells and 167 for HCT116 cells, LRP protein levels were significantly reduced in both cell types by treatment with TNF-α. The maximum effects were observed after 48 hours of treatment for HCT15 cells (mean LRP fluorescence/cell value = 67) and 72 hours of treatment for HCT116 cells (mean LRP fluorescence/cell value = 65) ($P = .0022$ for both cell types; Fig. 2, A). This modulation of LRP protein expression was also time dependent, consistent with the reduction in LRP mRNA that was induced by TNF-α and observed by means of RT–PCR.

In comparison with the MRP protein level in nontreated HCT116 cells (mean MRP fluorescence/cell value = 314), the MRP protein level in TNF-α-treated cells was significantly increased, with a mean MRP fluorescence/cell value of 662 after 12 hours of incubation ($P = .0022$) (Fig. 2, B). In HCT15 cells, however, MRP protein levels were not influenced by TNF-α incubation, again paralleling the mRNA data.

### LRP and MRP mRNA Expression in TNF-α-Secreting HCT15 and HCT116 Cell Clones

To evaluate the TNF-α-mediated modulation of LRP and MRP gene expression further, a plasmid vector harboring a human TNF-α cDNA was introduced into HCT15 cells and HCT116 cells, thus creating stably transfected, TNF-α-secreting clones as previously described (*11*). The following TNF-α-producing clones and the amounts of

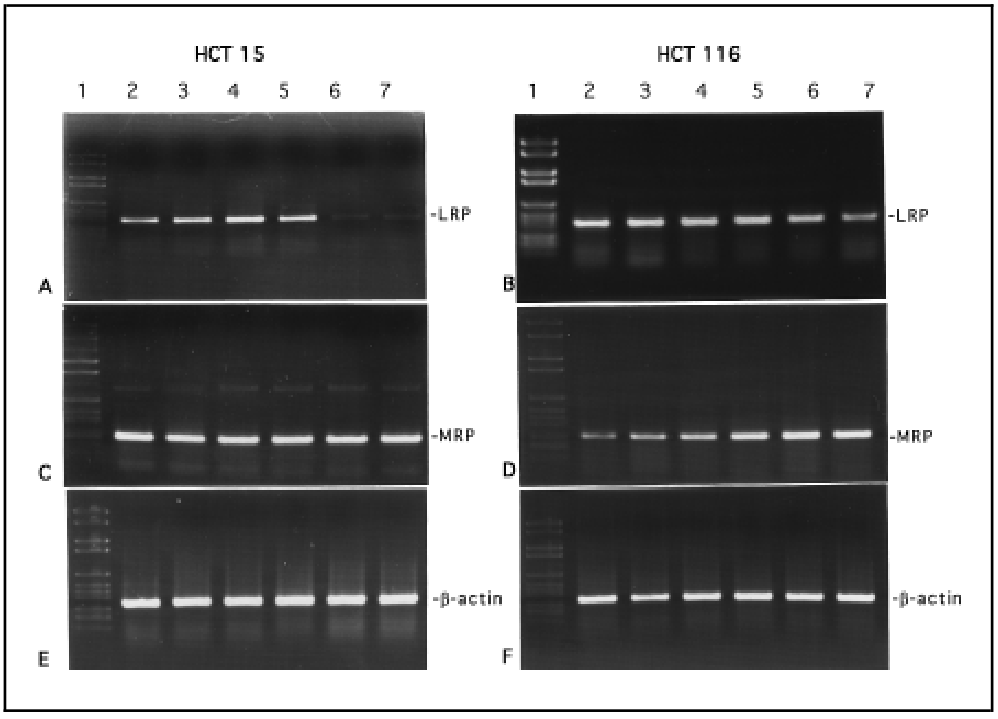


**Fig. 1.** Reverse transcription–polymerase chain reaction (RT–PCR) analysis of messenger RNA expression by genes encoding lung resistance protein (LRP) and multidrug resistance-associated protein (MRP) in the human colon carcinoma cell lines HCT15 and HCT116 following treatment with tumor necrosis factor-α (TNF-α). Cells were treated with TNF-α (100 U/mL) for 2, 12, 24, 48, or 72 hours. Untreated cells served as controls. Total cellular RNA was isolated and RT–PCR analysis was performed as described in the ''Materials and Methods'' section. The left panels (**A, C,** and **E**) show results obtained with HCT15 cells, and the right panels (**B, D,** and **F**) show results obtained with HCT116 cells. A and B = LRP-specific RT–PCR product (405 base pairs [bp]); C and D = MRP-specific RT–PCR product (291 bp); and E and F = β-actin-specific RT–PCR product (316 bp). Lane 1 = DNA molecular weight marker VI (Boehringer Mannheim GmbH, Mannheim, Germany); lane 2 = RT–PCR product from control cells; and lanes 3-7 = RT–PCR products for cells treated with TNF-α for 2, 12, 24, 48, or 72 hours, respectively.

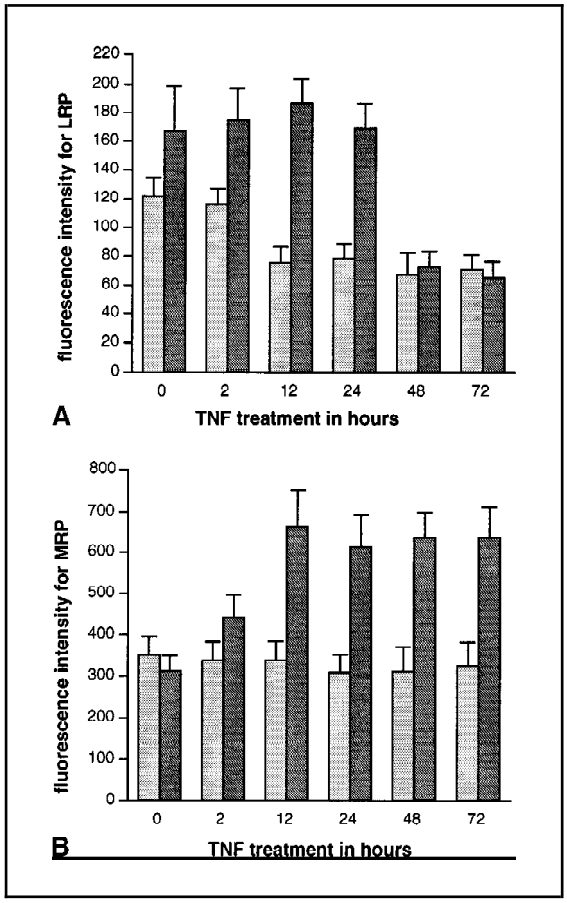


**Fig. 2.** Expression of lung resistance protein (LRP) and multidrug resistance-associated protein (MRP) in the human colon carcinoma cell lines HCT15 and HCT116 following treatment with tumor necrosis factor-α (TNF-α). Cells were treated with TNF-α (100 U/mL) for 2, 12, 24, 48, or 72 hours. Untreated cells served as controls. The cells were then exposed to monoclonal antibodies directed against LRP (antibody LRP-56) or MRP (antibody MRPr1), followed by exposure to fluorescein-conjugated secondary antibodies. The fluorescence intensity of $1 \times 10^4$ cells per group was measured with a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Each value represents the average fluorescence intensity of duplicate measurements from three independent experiments (standard deviations were <10% for LRP and <15% for MRP). TNF-α-mediated, time-dependent differences in mean fluorescence intensity were tested for significance with the two-sided Mann–Whitney rank sum test (nonparametric) (see text). **A** = fluorescence intensity for LRP and **B** = fluorescence intensity for MRP. Left columns at each time point show results obtained with HCT15 cells, and right columns show results obtained with HCT116 cells.

TNF-α secreted per milliliter of cell-conditioned medium were isolated: pM3CMV-hTNF-$1_{HCT15}$—1550 pg TNF-α/mL; pM3CMV-hTNF-$4_{HCT15}$—450 pg TNF-α/mL; pM3CMV-hTNF-$4_{HCT116}$—300 pg TNF-α/mL; and pM3CMV-hTNF-$5_{HCT116}$—500 pg TNF-α/mL (*see* Fig. 3, A and 3, B; fourth and fifth lanes). After normalization to β-actin mRNA levels (Fig. 3, G and H; fourth and fifth lanes) and comparison with LRP mRNA levels in parental, nontransfected cells (Fig. 3, C and D; second lanes) and empty vector (pM3neo)-containing cells (Fig. 3, C and D; third lanes), LRP gene expression, measured at the level of mRNA, was found to be reduced in the TNF-α-expressing clones of both cell lines (Fig. 3, C and D; fourth and fifth lanes). Although the RT–PCR method used in this study is semiquantitative at best, the decrease in LRP mRNA expression appeared to be related to the amount of TNF secreted by the particular cell clone.

In contrast, MRP mRNA expression was found to be increased in the TNF-α-expressing clones of HCT15 cells and HCT116 cells (Fig. 3, E and F; fourth and fifth lanes) when compared with the expression levels in nontransfected parental

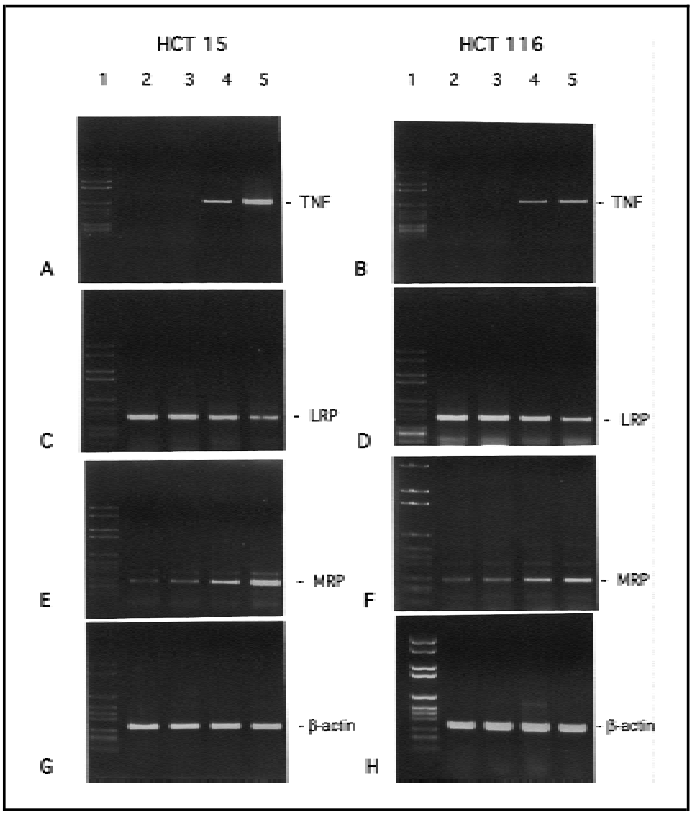


**Fig. 3.** Reverse transcription–polymerase chain reaction (RT–PCR) analysis of messenger RNA expression by genes encoding lung resistance protein (LRP) and multidrug resistance-associated protein (MRP) in human colon carcinoma HCT15 and HCT116 cells that express human tumor necrosis factor-α (TNF-α). Cells were transfected with the TNF-α expression vector pM3CMV-hTNF, and the clones pM3CMV-hTNF-$1_{HCT15}$ (secreting 1550 pg hTNF/mL [of cell-conditioned medium]), pM3CMV-hTNF-$4_{HCT15}$ (secreting 450 pg hTNF/mL), pM3CMV-hTNF-$4_{HCT116}$ (secreting 300 pg hTNF/mL), and pM3CMV-hTNF-$5_{HCT116}$ (secreting 500 pg hTNF/mL) were isolated. Nontransfected cells and cells transfected with the empty pM3neo vector served as controls. Total RNA was isolated and RT–PCR analysis was performed as described in the ''Materials and Methods'' section. The left panels (**A, C, E,** and **G**) show results obtained with transfected and nontransfected HCT15 cells, and the right panels (**B, D, F,** and **H**) show results obtained with HCT116 cells. A and B = TNF-α-specific RT–PCR product (702 base pairs [bp]); C and D = LRP-specific RT–PCR product (405 bp); E and F = MRP-specific RT–PCR product (291 bp); and G and H = β-actin-specific RT–PCR product (316 bp). Lane 1 = DNA molecular weight marker VI (Boehringer Mannheim GmbH, Mannheim, Germany); lane 2 = RT–PCR product from parental, nontransfected cells; lane 3 = RT–PCR product from pM3neo-transfected cells; lane 4 = RT–PCR product from clone pM3CMV-hTNF-$4_{HCT15}$ or clone pM3CMV-hTNF-$4_{HCT116}$, respectively; and lane 5 = RT–PCR product from clone pM3CMV-hTNF-$1_{HCT15}$ or clone pM3CMV-hTNF-$5_{HCT116}$, respectively.

cells or pM3neo-containing cells (Fig. 3, E and F; second and third lanes, respectively).

### LRP and MRP Protein Expression in TNF-α-Secreting HCT15 and HCT116 Cell Clones

To corroborate the data obtained for TNF-α-modulated mRNA levels, LRP and MRP protein levels were determined in the TNF-α-secreting clones of both cell lines. The clones pM3CMV-hTNF-$1_{HCT15}$, pM3CMV-hTNF-$4_{HCT15}$, pM3CMV-hTNF-$4_{HCT116}$, and pM3CMV-hTNF-$5_{HCT116}$ were incubated with either LRP-56 of MRPr1 primary antibodies and fluorescein-conjugated secondary antibodies as described above.

As shown in Fig. 4, A, LRP protein levels were reduced in all TNF-α-secreting clones of both cell lines in comparison with the levels found in parental cells or in pM3neo-containing cells. The maximum effects were observed with the clones that secreted the largest amounts of TNF-α, i.e., pM3CMV-hTNF-$1_{HCT15}$ and pM3CMV-hTNF-$5_{HCT116}$. This significant decrease in LRP protein expression ($P = .0022$ for both TNF-α-secreting clones) confirms the data described above for the TNF-α-mediated modulation of LRP mRNA levels. The extent of the reduction in LRP protein expression apparently depends on the amount of TNF-α secreted.

The level of MRP protein was also influenced by the secretion of TNF-α, but in the opposite manner. MRP protein was increased in the TNF-α-secreting clones of both cell lines (Fig. 4, B). In comparison with the MRP fluorescence levels in parental cells and in pM3neo-containing cells, the fluorescence levels were highest in the pM3CMV-hTNF-$1_{HCT15}$ and pM3CMV-hTNF-$5_{HCT116}$ clones, i.e., the ones that secreted the large amounts of TNF-α. These increases in MRP protein were statistically significant ($P = .0022$ for both TNF-α-secreting clones). Thus, TNFα-mediated modulation of MRP was not only observed in HCT116 cells, consistent with what was shown above in the experiments using externally added TNF-α, but also in the more drug-resistant HCT15 cells. Moreover, the data obtained with the TNF-α-transfected cells at the MRP mRNA level were consistent with the data obtained at the protein level.

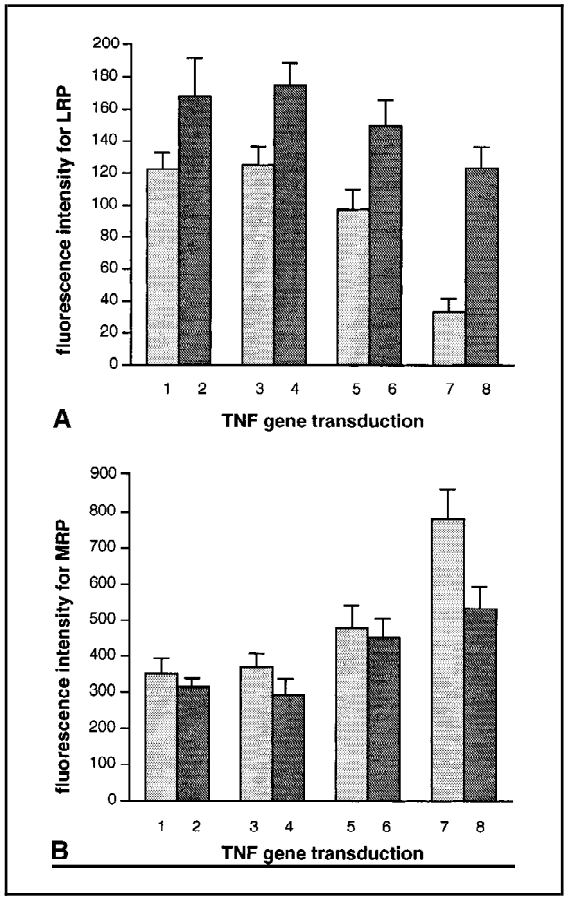


**Fig. 4.** Expression of lung resistance protein (LRP) and multidrug resistance-associated protein (MRP) in human colon carcinoma cells HCT15 and HCT116 that express human tumor necrosis factor-α (TNF-α). Cells were transfected with the TNF-α expression vector pM3CMV-hTNF, and the clones pM3CMV-hTNF-$1_{HCT15}$ (secreting 1550 pg TNF-α/mL [of cell-conditioned medium]), pM3CMV-hTNF-$4_{HCT15}$ (secreting 450 pg TNF-α/mL), pM3CMV-hTNF-$4_{HCT116}$ (secreting 300 pg TNF-α/mL), and pM3CMV-hTNF-$5_{HCT116}$ (secreting 500 pg TNF-α/mL) were isolated. Nontransfected cells and cells transfected with the empty pM3neo vector served as controls. The cells were incubated with monoclonal antibodies directed against LRP (antibody LRP-56) or MRP (antibody MRPr1), followed by exposure to fluorescein-conjugated secondary antibodies. The fluorescence intensity of $1 \times 10^4$ cells per group was measured with a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Each value represents the average fluorescence intensity of duplicate measurements from three independent experiments (standard deviations were <10% for LRP and <15% for MRP). TNF-α-mediated differences in mean fluorescence intensity were tested for significance with the two-sided, Mann–Whitney rank sum test (nonparametric) (see text). **A** = fluorescence intensity for LRP, and **B** = fluorescence intensity for MRP. The numbered columns identify results obtained with the following cell types: 1 = parental, nontransfected HCT15 cells; 2 = parental, nontransfected HCT116 cells; 3 = pM3neo-transfected HCT15 cells; 4 = pM3neo-transfected HCT116 cells; 5 = clone pM3CMV-hTNF-$4_{HCT15}$; 6 = clone pM3CMV-hTNF-$4_{HCT116}$; 7 = clone pM3CMV-hTNF-$1_{HCT15}$; and 8 = clone pM3CMV-hTNF-$5_{HCT116}$.

## Discussion

We have shown that TNF-α treatment influences LRP and MRP gene expression in distinctly different ways. The pattern of response for LRP gene expression is very similar to that which we have previously reported for the P-glycoprotein gene (i.e., MDR-1) expression (*11*). At both the mRNA and protein levels, exposure of multidrug-resistant human colon carcinoma cells to exogenous TNF-α resulted in a dramatic, dose- and time-dependent reduction in LRP expression. The time–course data indicate that TNF-α treatment can induce relatively rapid (<12 hours for HCT15 cells) reductions in LRP protein levels. Since these changes precede reductions in mRNA levels, it appears that TNF-α may have an effect on the stability or turnover of LRP protein or of cytoplasmic vault particles. Additional studies will be required to define this phenomenon. In a preliminary evaluation of LRP mRNA transcription rates (using a nuclear runoff assay), we have found further that highly drug-resistant HCT15 cells exhibit a dramatic reduction in LRP gene transcription after 72 hours of TNF-α treatment. When the data were normalized with respect to γ-actin gene transcription and compared with the basal level of transcription in HCT15 cells, we concluded that the reductions in LRP mRNA in these cells were a consequence of substantially reduced transcription (mean relative rates: 0.460 for untreated cells versus 0.165 for TNF-α-treated cells; determined in two independent experiments). HCT116 cells exhibited a very low basal rate of LRP transcription, and no significant change was observed following TNF-α treatment despite a clear reduction in LRP mRNA and protein expression levels. This lack of a substantial change in transcription may be a consequence of the very low basal rate of

LRP gene expression in these cells, or it may reflect an effect of TNF-α on mRNA stability or processing in the HCT116 cell line. Transfected tumor cell populations yielded data similar to those obtained at longer time points with exogenous TNF-α treatment. This closer correspondence between mRNA and protein levels may reflect effects of TNF-α occurring at the transcriptional level. Since we have previously demonstrated that reductions in MDR-1 mRNA and P-glycoprotein levels produced by TNF-α treatment in these cell populations are associated with increased drug accumulation and increased cytotoxic responses, it is likely that the same association holds true for LRP.

In the case of MRP, a nearly opposite effect of TNF-α was observed. Except in the situation where exogenous TNF-α treatment was observed to have no effect on MRP expression in HCT15 cells, exposure to TNF-α was generally found to produce dose- and time-dependent increases in MRP expression. Since these effects were observed under conditions that are known to increase cellular sensitivity to classical multidrug resistance-associated drugs, such as doxorubicin and vincristine, it would appear that the role of MRP in mediating resistance to these drugs is offset by the action of MDR-1- and LRP-mediated effects. This conclusion is consistent with clinical findings in both solid (*26*,*27*) and hematologic (*28*) cancers, indicating that LRP, but not MRP, overexpression predicted reduced responsiveness to chemotherapy and/or clinical outcome, thereby underlining the potential importance of LRP as an additional drug resistance-associated gene.

The observation that a single cytokine can have effects on the expression of multiple genes is not new. It seems likely that the effects of TNF-α described herein may result from the activation/deactivation of a signal-transduction or a transcription-factor cascade [(e.g., *10*,*38-40*)]. However, while considerable information is available about the MDR-1 gene promoter [e.g., (*41-45*)] and some information has more recently become available about the MRP promoter (*46*,*47*), nothing is currently known regarding the LRP promoter. Our results provide further support for the view that the LRP and MRP genes, although concomitantly overexpressed in many multidrug-resistant tumor cell lines and co-localized on chromosome 16, do not belong to the same amplicon (*48*). Certainly, a formal genetic analysis of the promoters of these genes and their associated transcription factors could aid in the understanding of the apparent pleiotropic effects of TNF-α.

The pleiotropic features of multidrug resistance have been recognized since the earliest reports of the phenomenon (*49-51*). It now appears that multiple genes with pleiotropic effects are important in the multidrug-resistant phenotype, and therapeutic strategies designed to circumvent this phenotype will need to address this situation. The possibility exists that certain treatments, including those targeted at a single specific multidrug resistance mechanism, may have anticomplementary effects on other mechanisms. In the case of TNF-α modulation of multidrug resistance, the apparently favorable effects on two drug resistance-associated genes are associated with an increased effectiveness of drug treatment. This result supports the notion that some strategies may have a net effect that can be used to therapeutic advantage. Further development of gene therapy-type approaches that can minimize the systemic toxic effects of TNF-α and other cytokines may offer attractive future directions for this line of research.

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## Notes

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