

EFFECT OF INTERFERON- γ AND TNF- α ON MUC1 MUCIN EXPRESSION IN OVARIAN CARCINOMA CELL LINES

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SUMMARY

In view of the potential uses of cell surface tumour associated antigens in novel anticancer treatment, a study was designed to investigate whether the biological response modifiers interferon-gamma (IFN- γ) and tumour necrosis factor-alpha (TNF- α) could effect the expression of an epitope on the tumour associated MUC1 epithelial mucin. Four ovarian carcinoma cell lines showing high (OAW42 and GG) and low (JAM and PE01) basal expression of MUC1 were treated with 10-1000 U/mL of IFN- γ or TNF- α for one or five days. Changes in MUC1 expression in cells exposed to IFN- γ or TNF- α were monitored using an ELISA technique with the monoclonal antibody BC2 which reacts with a core protein epitope on the MUC1 mucin, and then corrected for the number of viable cells present. TNF- α had little effect on MUC1 expression, but one or five days exposure to IFN- γ significantly increased MUC1 expression ($p < 0.01$) in all cell lines including the two cell lines that initially showed little or no expression.

KEYWORDS: Interferon- γ Tumour necrosis factor- α MUC1 Mucin Ovarian cancer

INTRODUCTION

Despite widespread use of platinum based chemotherapy less than 25% of patients with advanced ovarian carcinoma survive more than 5 years (Annual Report of the Results of Treatment of Gynecological Cancer, 1990). Consequently, there is a critical need for new approaches to the management of this disease. Biological response modifiers, including tumour necrosis factor alpha (TNF- α) and interferon-gamma (IFN- γ), have shown promise as anticancer agents due to their immunomodulatory and cytotoxic actions.

TNF- α has not been shown to modulate tumour associated antigen (TAA) expression (Marth *et al.*, 1989), however it can alter expression of Class I (Collins *et al.*, 1986) and

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Class II MHC antigens (Chang and Lee, 1986). In addition to inducing the production of proteins encoded by the MHC class I and II regions (Chang and Lee, 1986), *in vitro*, *in vivo* and clinical studies have indicated that interferons can modulate the cell surface expression of tumour associated antigens (TAA) (Basham *et al.*, 1986; Borden, 1988; Greiner *et al.*, 1987) including the MUC1 epithelial mucin (Greiner *et al.*, 1984; Tran *et al.*, 1988).

The epithelial mucin encoded by the MUC1 gene (Gendler *et al.*, 1991) has been used as a target for antibody-directed immunotherapy and immunoscintigraphy because of its generally high level of cell surface expression in breast and ovarian cancers and the availability of antibodies reactive with various MUC1 epitopes (Taylor-Papadimitriou *et al.*, 1993). In addition, both T cell (Ioannides *et al.*, 1993; Barnd *et al.*, 1989; Jerome *et al.*, 1991) and B cell (Rughetti *et al.*, 1993) immune responses to this molecule have been demonstrated in patients with ovarian and breast cancer. Therefore adoptive immunotherapy may be employed in the future using autologous lymphocytes recognizing MUC1 epitopes. However, there is considerable heterogeneity of expression of MUC1 both within and between cancers (McGuckin *et al.*, 1991). Exploring the potential of biological agents to modulate the expression of MUC1 is of considerable interest as by increasing tumour expression of these target molecules, antibody-directed therapy may become practicable. This would be particularly advantageous if the biological agents also possessed anti-tumour activity themselves. This study aimed to investigate whether IFN- γ or TNF- α could effect the expression of a peptide epitope on MUC1 in ovarian cancer cells showing high and low levels of basal expression.

MATERIALS AND METHODS

Cell lines and culture

Four continuous human ovarian carcinoma cell lines, selected to provide a range of basal expression of MUC1, were used for *in vitro* experimentation. These cell lines were all derived from serous cystadenocarcinomas of the ovary: GG (Van Haaften Day *et al.*, 1983), OAW42 (Wilson *et al.*, 1984), JAM (Ward *et al.*, 1987) and PEO1 (Langdon *et al.*, 1988). Cell cultures were maintained in either 25, 75 or 150 cm² sterile tissue culture flasks (Corning, USA) at 37°C in a humidified atmosphere of 5% CO₂ in RPMI1640 containing 10% foetal calf serum and antibiotics (penicillin 100 u/mL, streptomycin 100 ug/mL and amphotericin B 0.25 ug/mL; Flow Laboratories, UK). Once every four weeks cells were cultured in an antibiotic-free environment. Cultures were passaged at confluence by trypsinisation.

Biological response modifiers

E. coli derived recombinant human tumour necrosis factor [TNF- α : lot # L26105 G9674, endotoxin (LAL): < 1 EU/mg, purity 99%] was a gift from Boehringer Ingelheim P/L, Australia. Lyophilised TNF- α was reconstituted in Dulbecco's phosphate buffered saline (dPBS) containing 3 mg/mL bovine serum albumin (Sigma, USA) (Dealtry and Balkwill, 1987). Aliquots of 1 mL containing 1×10^5 U/mL were then stored at -70°C until use. Recombinant human γ interferon [RU 42 369: IFN- γ batch # L400, control # 9S 0493F, endotoxin: < 0.24 EU/mg, purity > 95%] was a gift from Roussel Uclaf, France. Lyophilised IFN- γ was reconstituted in RPMI 1640 containing 10% FCS. Aliquots of 1×10^5 U/mL were then stored at -70°C until use.

Cell surface antigen expression assay

Levels of cell surface MUC1 expression were determined by a micro-ELISA technique incorporating the monoclonal antibody BC2 (Xing *et al.*, 1989) to detect the MUC1 antigen. Ovarian cancer cell lines were harvested, counted and plated in 96 well flat-bottomed microtitration tissue culture plates (Nunc) in 100 μL of RPMI 1640 media with 10% FCS. Plates were incubated for 4 h to permit adherence of cells. Various dilutions of IFN- γ and TNF- α in tissue culture media were added to wells giving final concentrations ranging from 0 to 1000 U/mL in a total volume of 200 μL . Control wells received 100 μL of drug-free media. Experiments were conducted using quadruplicate wells. A second plate was prepared in an identical manner to serve as a control for any variation in cell number that might occur due to IFN- γ or TNF- α . Cells were incubated for either 24 h or 5 days in the presence of IFN- γ and TNF- α . Experiments were designed such that control wells were confluent at completion of the incubation period. This meant seeding 24 h plates at 5×10^4 cells per well, and 5 day plates at either 1×10^3 cells per well or 4×10^3 cells per well depending on whether the cell line had high or low doubling time, respectively.

Following treatment the media was tipped off the plate and the cells fixed with methanol for 10 minutes. Prior to ELISA, cells were stored in 0.1% gelatin in dPBS at 4°C. The ELISA was performed using the method of McGuckin *et al.* (1990a). The optical density using a negative control monoclonal antibody of the same isotype was subtracted from the optical density using the BC2 antibody. The second plate was used to correct for any variation in cell number that might result due to any cytotoxic, growth inhibitory or growth stimulatory effects of the IFN- γ or TNF- α . The neutral red assay (see below) was used to quantitate cell number as this assay demonstrates a linear relationship between cell number and dye uptake (Parish and Mullbacher, 1983). Antigen expression, in OD units, was then corrected for deviation in cell number from the control for each cell line.

Neutral red cytotoxicity assay

A slight modification of the Neutral Red Assay described by Parish and Mullbacher (1983) was then performed. Briefly, at the end of the incubation plates were centrifuged (5 min, 600g), 100 μL of 0.036% neutral red in Hanks buffered salt solution was added to each well and plates incubated for a further 30 min at 37°C. Solution was gently blotted from the plates and plates washed twice with 200 μL of RPMI1640 with 1% FCS, then 200 μL of 1:1 0.1 M acetic acid:ethanol added to each well and plates rocked for 5 min before reading OD 540nm in a Multiskan plate reader (Flow Laboratories, UK).

Statistics

Statistical differences between treatment groups were assessed using ANOVA and the Duncans Multiple Range test using the SAS STAT 6.04 program (SAS Institute, USA) on a personal computer.

RESULTS

The basal levels of cell surface expression of the MUC1 epithelial mucin in the four cell lines are shown in Table 1. The cell lines OAW42 and GG highly expressed the MUC1 antigen, while the cell lines PEO1 and JAM showed weak and undetectable expression of MUC1, respectively.

Table 1. Cell Surface expression of the MUC1 epithelial mucin in four ovarian carcinoma cell lines.

Cell line	OD 405 nm ^a
OAW42	1.119 ± 0.039
GG	0.840 ± 0.051
PEO1	0.133 ± 0.024
JAM	0.005 ± 0.015

a. Mucin expression was quantitated by ELISA using antibody BC2, the mean ± s.d. is shown after subtraction of OD405 nm using a negative control antibody.

Figure 1a illustrates the level of MUC1 expression in each cell line following 24 h exposure to 0, 10, 100 and 1000 U/mL of IFN- γ . Data are presented as OD units after correction for cell number relative to control wells. A significant increase in MUC1 expression occurred with exposure of all cell lines to IFN- γ despite differing levels of antigen expression in each cell line prior to drug exposure. Dose dependent increases in MUC1 expression were seen, with even the lowest dose of IFN- γ (10 U/mL) significantly increasing expression in the PEO1, GG and OAW42 cell lines.

Figure 1b illustrates the levels of MUC1 expression in each cell line following 24 h exposure to 0, 10, 100 and 1000 U/mL of TNF- α . Data are presented as OD units after correction for cell number relative to control wells. In contrast to IFN- α , TNF- γ failed to significantly alter MUC1 expression in all cell lines except for small increases in PEO1 cells.

Similar antigen modulation experiments were conducted using a 5 day incubation period with each agent at concentrations of 0, 10 and 1000 U/mL. Results reflected trends outlined in the 24 h experiments. Incubation with IFN- γ for 5 days caused significant increases ($p < 0.01$) in MUC1 expression in all cell lines; the magnitude of these increases being similar to that observed following 24 h exposure. Five days exposure to TNF- α did not alter the expression of MUC1 in OAW42 and GG cells, and stimulated small increases in MUC1 expression in PEO1 and JAM cells ($p < 0.05$, data not shown).

DISCUSSION

Although no truly specific markers of ovarian carcinoma have as yet been reported, a number of TAA have been described which occur on ovarian cancer cells to a far greater extent than other tissue. The mucin produced by the MUC1 gene, as detected by the BC2 monoclonal antibody, is found in serum and on tumour cells of many patients with ovarian carcinoma (McGuckin *et al.*, 1990b, 1991). Cell lines in this study demonstrated a range in basal levels of MUC1 expression. This range of antigen expression reflects clinical findings in which variation in MUC1 expression occurs between tumours. TNF- α possessed only limited and variable activity in modulating the expression of MUC1.

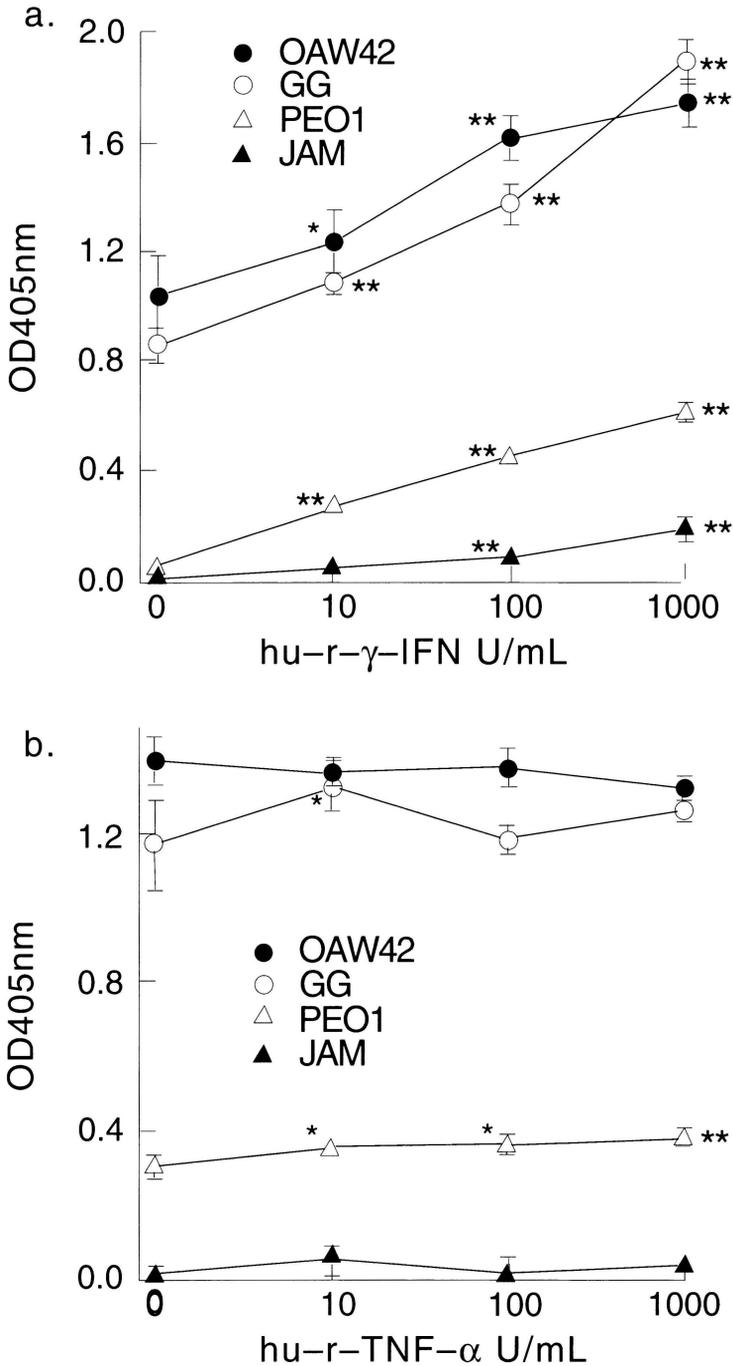


Figure 1. Modulation of MUC1 expression by 24 h exposure to IFN- γ (a) and TNF- α (b). MUC1 expression was quantitated by ELISA using antibody BC2 and the OD405 nm corrected for the OD405 nm using a negative control antibody and the cell number relative to control wells. Statistics: mean \pm s.d.; ANOVA: * p < 0.05 vs control, ** p < 0.01 vs control.

Although TNF- α has not been found to modulate TAA expression in another study (Marth *et al.*, 1992), the small increases in one cell line in these experiments are not totally unexpected given the agent's reported ability to augment the expression of other cell surface molecules, such as Class I and Class II MHC antigens (Collins *et al.*, 1986; Chang and Lee, 1986). Furthermore, one *in vivo* study has shown that TNF- α can increase the localisation of certain labelled monoclonal antibodies to tumours (Smyth *et al.*, 1988). Nevertheless, changes in MUC1 expression induced by TNF- α in this series of experiments suggests TNF- α is of little value in inducing substantial, reproducible and predictable changes in MUC1 expression across a spectrum of ovarian cancer cell phenotypes.

In contrast to TNF- α , IFN- γ was a powerful modulator of MUC1 expression *in vitro*. Dose dependent modulation of antigen expression was demonstrated across all cell lines. Increases of MUC1 expression reached a maximum at a dose of 1000 U/mL and within 24 hs of drug exposure and these increases were maintained over a 5 day exposure. Importantly, significant increases in MUC1 expression occurred in all cell lines (and in particular, in cell lines weakly expressing (PE01) and showing no detectable expression (JAM) at low and clinically achievable doses of IFN- γ (10 - 100 U/mL) (Kuzrock *et al.*, 1985; Willemse *et al.*, 1990). These results support previous reports of modulation of the expression of a number of TAA including MUC1 by IFN- γ *in vitro* (Greiner *et al.*, 1984; Tran *et al.*, 1988). However, Tran *et al.* (1988) found that breast cancer cell lines that did not express detectable levels of four TAA remained negative after treatment with interferon. However, all the cell lines in this study did show detectable basal levels of MUC1 core protein epitopes as detected by the DF3 antibody. These data are consistent with previous studies demonstrating that although increased TAA expression is maintained with extended exposure of interferon, maximum modulation in TAA expression occurs within 24 hs of exposure (Greiner *et al.*, 1987; Marth *et al.*, 1989).

These *in vitro* studies indicate that IFN- γ increases ovarian tumour cell expression of the MUC1 mucin, even in cells showing very low basal expression, and may facilitate use of this antigen as a target for immunoconjugate and adoptive immunotherapy.

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