

## INFLUENCE OF RECOMBINANT INTERFERON-GAMMA ON THE EXPRESSION OF MHC CLASS I AND CLASS II ANTIGENS ON FOUR HUMAN COLONIC CARCINOMA CELL LINES.

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### Summary

The occurrence of MHC class I and class II antigens on four human colonic carcinoma cell lines and the effect of recombinant interferon-gamma (rIFN $\gamma$ ) on the expression of these antigens was investigated by immunofluorescent flow cytometry. The concentration of rIFN $\gamma$  which resulted in the largest increase in expression of class I and class II antigens was determined. Changes in the amount of MHC antigen on the membrane were indicated by a shift in the mean fluorescence intensity (MFI) of the cell population. Without addition of rIFN $\gamma$ , the COLO 206, COLO 320F and COLO 397 cell lines were class I positive although the COLO 206 cell line expressed less class I antigen than the other two lines. The HT-29 cell line expressed only a minimal level of class I antigen. Treatment with rIFN $\gamma$  increased the amount of class I antigen on these cell lines 5, 1.4, 2.5 and 20 times respectively. Maximum levels of class I antigen were found two days after treatment. Class I antigen expression returned to pre-treatment levels by day 8 in all but the HT-29 cell line, which maintained its increased level following a single dose of rIFN $\gamma$ . All four cell lines had little or no class II antigens. Following treatment with rIFN $\gamma$ , DR antigen appeared on all four lines whereas DP and DQ antigens could be induced only on the 320F and 397 lines. The amount of class II antigen reached its peak two days after treatment and gradually decreased over the next 6 days of culture. These findings are discussed in the light of previous immunohistochemical studies on the occurrence of class I and class II antigens in colonic carcinomas. These antigens may play a role in tumour recognition.

Keywords: Colonic carcinoma cell lines, interferon-gamma, MHC antigens, mean fluorescence intensity.

### INTRODUCTION

Numerous studies have alluded to the importance of class I and class II MHC antigen expression by tumours in the development of anti-tumour immunity. It has been clearly established that both quality<sup>1</sup> and quantity<sup>2</sup> of MHC antigens expressed by target cells can influence the efficacy of immune reactions against these cells. Tumours can differ markedly from corresponding normal tissues in their expression of MHC antigens. Our<sup>3</sup> and other<sup>4,5</sup> observations on colonic carcinomas have shown that HLA class I antigen can be reduced or absent from some tumours when compared to normal colonic epithelium. In contrast, while normal colonic epithelium lacks class II antigens,<sup>6,7</sup> some class II antigens are expressed on colonic carcinomas where they appear more frequently on tumours of poor prognosis.<sup>3</sup> Furthermore, the 3 class

II antigens do not appear as a unit and DQ antigen is rarely expressed by colonic carcinomas.<sup>3,7</sup>

Since tumours are often surrounded by T-lymphocytes, the expression of MHC antigens by these tumours may be affected by products of these cells such as interferon-gamma (IFN $\gamma$ ). IFN $\gamma$  has been shown to modulate the expression of both class I and class II antigens on a variety of cell lines including melanomas<sup>8</sup> and colonic carcinomas.<sup>9,10</sup> This study examined four human colonic carcinoma cell lines for their expression of class I and the DR, DP and DQ class II antigens. In particular we examined the influence of recombinant interferon-gamma (rIFN $\gamma$ ) on the amount of these antigens expressed on the cell membrane. We discuss the possible role of IFN $\gamma$  in the expression of MHC antigens by colonic carcinomas.

## MATERIALS AND METHODS

### Colonic carcinoma cell lines

HT-29 was obtained from the American Type Culture Collection. COLO 206, COLO 320F and COLO 397 were developed by Dr. George Moore (Department of Health and Hospitals, Denver General Hospital, Colorado, USA) and received from Dr. Chris Thompson (University of Melbourne, Australia). The cells were grown at 37°C in a humidified atmosphere of 5% CO<sub>2</sub> in RPMI 1640 containing glutamine, penicillin, streptomycin and 10% foetal calf serum. Cultures were split weekly and, where necessary, cells were first dislodged by treatment with trypsin-EDTA before passage. When the immunofluorescence test was to be carried out, only EDTA was used to dislodge the cells. Preliminary tests carried out on the non-adherent cell line, COLO 320F, showed that EDTA did not affect either the viability or the detection of the antigens by monoclonal antibodies used in this study. Cell viability was determined by trypan blue exclusion.

### Treatment with rIFN $\gamma$

The optimal concentration of rIFN $\gamma$  was first determined by growing the cells in the presence of various concentrations of rIFN $\gamma$  (50 U/ml to 1000 U/ml) (Amersham, UK.) and measuring the amounts of class I and class II antigens on the cells on day 2 of culture. The highest staining intensities were obtained with 100 U/ml on COLO 206 and COLO 397, 50 U/ml with HT-29 and 200 U/ml with COLO 320F. These optimal concentrations of rIFN $\gamma$  were added to each cell line at the start of culture and the cell lines were allowed to grow for the whole 8 days of the experiment without passaging.

### Monoclonal antibodies

The monoclonal antibodies<sup>11,12,13,14,15</sup> used and their sources are listed in Table 1. Tu22 was a gift from Dr. A. Ziegler, Tubingen, West Germany and B7/21 was from Dr. I. Trowbridge, The Salk Institute, San Diego, California.

### Immunofluorescence and flow cytometry analysis

The expression of the various antigens was detected by indirect immunofluorescence and analysed in the FACS Analyser (Becton Dickinson, Mountain View, CA). Cell

concentrations of between 1 X 10<sup>5</sup> to 5 X 10<sup>5</sup> per 50 ul were used for each test. An equal amount of hybridoma culture supernatant containing the appropriate monoclonal antibodies or a specified amount of commercially prepared purified monoclonal antibodies was added. Hybridoma culture supernatants containing monoclonal antibodies with irrelevant or unknown specificities were used as isotype controls. Incubations were carried out for 30 min at 4°C. After washing, a fluorescein-conjugated goat anti-mouse immunoglobulin was added for a further 30 min at 4°C and the cells washed twice in PBS containing 0.02% sodium azide. Analysis of stained cells was carried out after first gating for live cells. Flow cytometer histograms were defined as plots of fluorescence intensity (X-axis) versus the number of cells counted (Y-axis). The mean fluorescence intensity (MFI) was determined using a Consort 30 computer system which was integrated with the FACS Analyser.

TABLE 1  
LIST OF MONOCLONAL  
ANTIBODIES USED

Antibody designation	Specificity	Source
W6/32	HLA-A, -B, -C (11)	ATCC
FMC 14	Class II monomorphic (12)	FMC
L243	HLA-DR (13)	BD
B7/21	HLA-DP (14)	Dr. I. Trowbridge
Tu 22	HLA-DQ (IS)	Dr. Ziegler

FMC = Flinders Medical Centre

(Dept. Clinical Immunology)

ATCC = American Type Culture Collection

BD = Becton Dickinson, California, USA

## RESULTS

Figure 1 presents the flow cytometry histograms of immunofluorescent staining patterns of the four cell lines after 2 days in culture with (light line) and without (heavy line) rIFN $\gamma$ . Figures 2a and 3a show the kinetics of expression of class I and class II antigens respectively. The data in Figures 2a and 3a are presented as the intensity of fluorescence (MFI) which was assayed before addition of rIFN $\gamma$  (day 0) and 2, 4, 6 and 8 days after treatment. Figures 2b and 3b summarise these results and show the MFI of untreated cells (open bars) and treated cells (hatched bars) at day 2.

**Expression of class I antigen**

The untreated COLO 206, 320F and 397 cell lines were class I positive, whereas only a very low level of fluorescence was detected on the HT-29 cell line. A single dose of rIFN $\gamma$  increased the amount of class I antigen 5, 1.4, 2.5 and 20 times on the 206, 320F, 397 and HT-29 cell lines respectively. Expression of class I antigen was seen at maximal levels on day 2 after a single dose of rIFN $\gamma$ . The amount of class I antigen gradually diminished and by day 8 had returned to pre-stimulation levels on the 206, 320F and 397 cell lines, whereas the peak level of expression seen on day 2 in the HT-29 cell lines was maintained to day 8.

**Expression of class II antigens**

Prior to treatment with rIFN $\gamma$ , there were

only small amounts of DR antigen on 206 cell lines while 320F and 397 cell lines expressed small amounts of DR, DP and DQ antigens. HT-29 did not express any of the class II antigens. DR antigen expression was either induced or enhanced on all four lines following a single dose of rIFN $\gamma$ , while DP and DQ could only be detected in appreciable amounts on the 320F and 397 cell lines; COLO 206 expressed only a very low level of DP. The MFI levels reached their peaks at day 2 (day 4 with DR antigen on HT-29) and gradually diminished by day 8 to levels near that seen prior to treatment with rIFN $\gamma$ . Expression of DR decreased least on the HT-29 cell line between day 2 and 8.

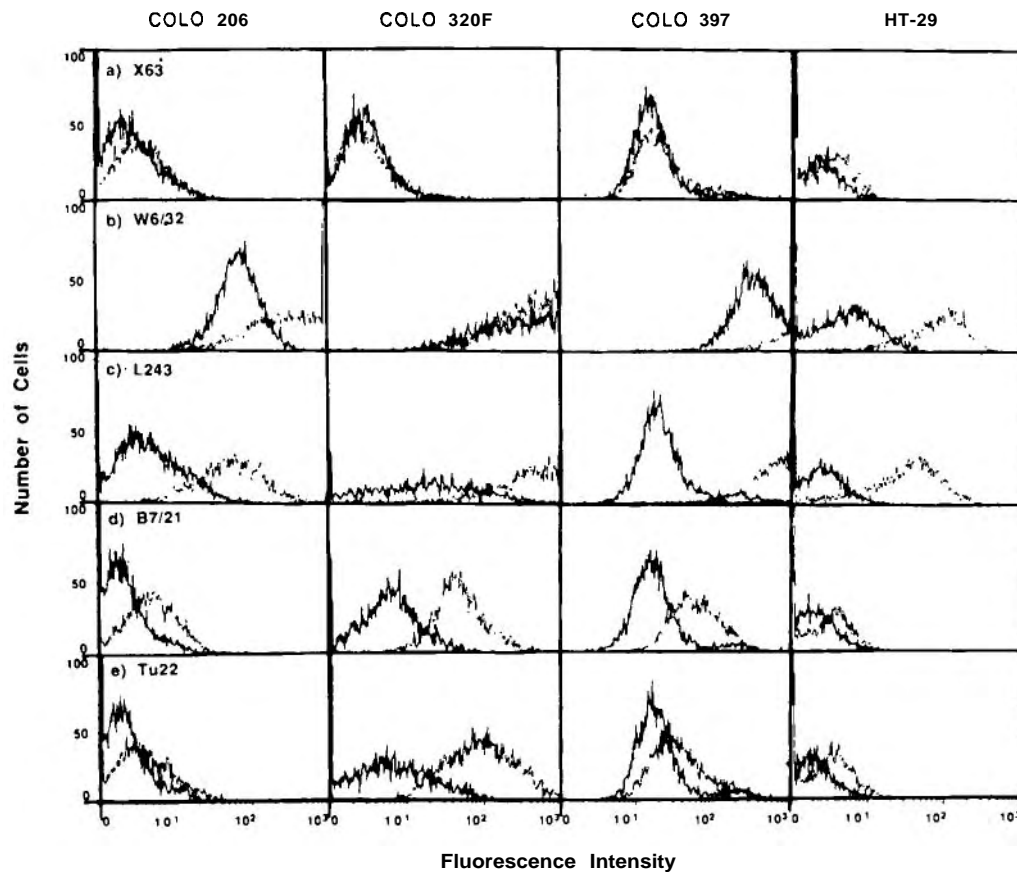


FIG. 1: Immunofluorescent flow cytometry of four human colonic carcinoma cell lines stained for class I antigen (W6/32) and the class II antigens DR (L243), DP (B7/21) and DQ (Tu22) before (heavy line) and after (light line) treatment with rIFN $\gamma$ . The results obtained using a control monoclonal antibody with unknown specificity, X63, was included for comparison.

**DISCUSSION**

*Data Presentation*

In order to highlight the effect of rIFN $\gamma$  treatment on the amount of MHC antigen expressed by tumour cells, we have presented our results as the intensity of staining for a particular antigen rather than as the percentage of positive cells as is more commonly done. These colonic carcinoma cell lines behave as a uniform population in that there is no bimodal distribution of fluorescence intensities. The change that is observed following treatment with rIFN $\gamma$  is a shift in the MFI of the cell population which is essentially described as a single peak of intensities. As such it is not appropriate to refer to positive and negative cells. We have used the MFI to report the findings of this study since this should reflect the number of accessible HLA molecules on the cell surface. The importance of the amount of HLA on the cell surface is found in studies which showed that not only were class I antigens required to be present on the tumours for animals to be able to mount efficient immune responses against a tumour, but that the efficiency of these responses was directly related to the number of the class I antigen molecules expressed by the tumour cell;<sup>2</sup> that is, tumour cell clones which expressed the largest amount of class I antigen were rejected more rapidly than those which expressed less.

*Expression of class I antigen*

While MHC class I antigens are found on the normal colonic epithelium, their occurrence on colonic carcinomas is irregular. These tumours have been reported to have a general decrease in the amount of class I antigens expressed or, in some individual cases, completely lack class I antigens, while other cases showed no change from the amount found on the normal epithelium.<sup>3,4,6</sup> This variability in tumour specimens is reflected in the cell lines used in this study and in another investigation.<sup>9</sup> In our study, while 3 of the cell lines were class I antigen positive, the fourth expressed only a very small amount of class I antigen. This fourth cell line (HT-29) was, however, able to express class I antigen on its surface following treatment with rIFN $\gamma$ . This treatment also increased the amount of class I antigen expressed by the other cell lines. Thus rIFN $\gamma$  in this study has been able to act as both an inducer and enhancer of the expression of class I antigen.

*Expression of class II antigen*

Immunohistochemical studies have shown that class II antigens are not detected on normal colonic epithelium but are found on colonic carcinomas.<sup>3,4,7</sup> Our previous findings<sup>3</sup> derived from analyses of tumour sections indicated that class II antigens were found more frequently on tumours with the poorest prognosis and the expression of the

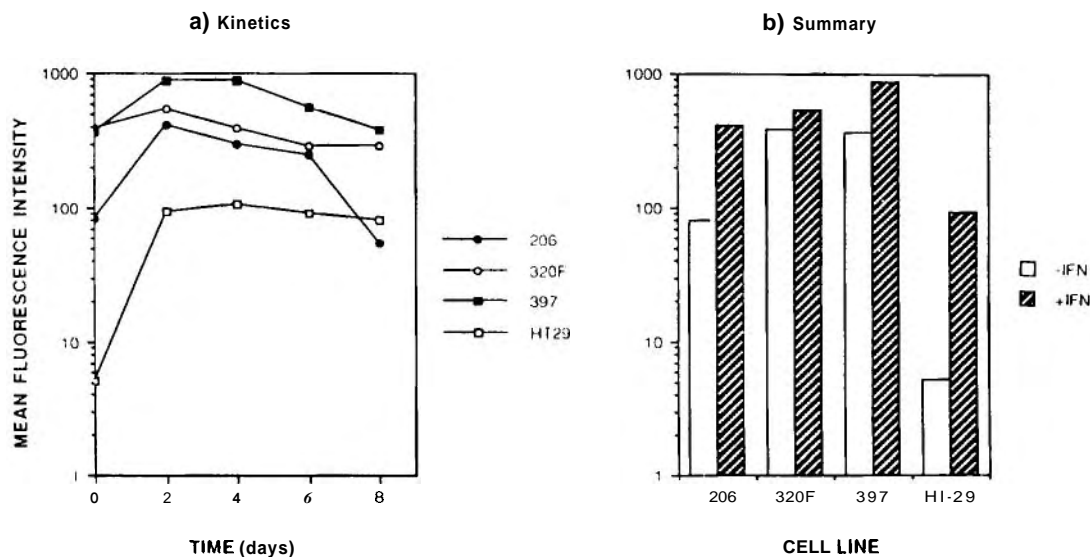


FIG. 2: Levels of expression of class I antigens by four human colonic carcinoma cell lines after treatment with rIFN $\gamma$ . (a) shows the kinetics of expression and (b) summarises the data from day 2, when expression was at its highest level. The open bars are untreated cultures and the hatched bars are cultures treated with rIFN $\gamma$ .

three class II gene products did not occur as a unit; that is, DR was expressed in more cases than DP which was in turn expressed more frequently than DQ.<sup>3,7</sup> The four cell lines examined in this study all lacked class II antigens. Following treatment with rIFN $\gamma$ , the response was varied in that only the 320F and 397 cell lines expressed all 3 class II antigens. While DR antigen could be found on the remaining 2 lines, DP and DQ antigens were absent. These results highlight the fact that these three D region gene products are not expressed uniformly on the cell surface as has been found in other studies on other tissues<sup>6,17</sup> or with other cell lines.<sup>8,18</sup>

DQ antigen appeared least inducible of the class II antigens. Using a different monoclonal antibody to detect DQ (Leu-10), Pfizenmaier<sup>10</sup> could not detect this antigen on four other colonic carcinoma cell lines following treatment with IFN $\gamma$ . Our results are in disagreement with those of Sollid *et al.*<sup>19</sup> who found that the HT-29 cell line expressed DQ and low levels of DP. We found neither antigen was expressed on this cell line, despite the fact that the dose of rIFN $\gamma$  and the monoclonal antibodies used in the two studies were the same. However, Sollid *et al.* did find that DQ antigen appeared later and reached a maximum level later than did DR or DP antigens.

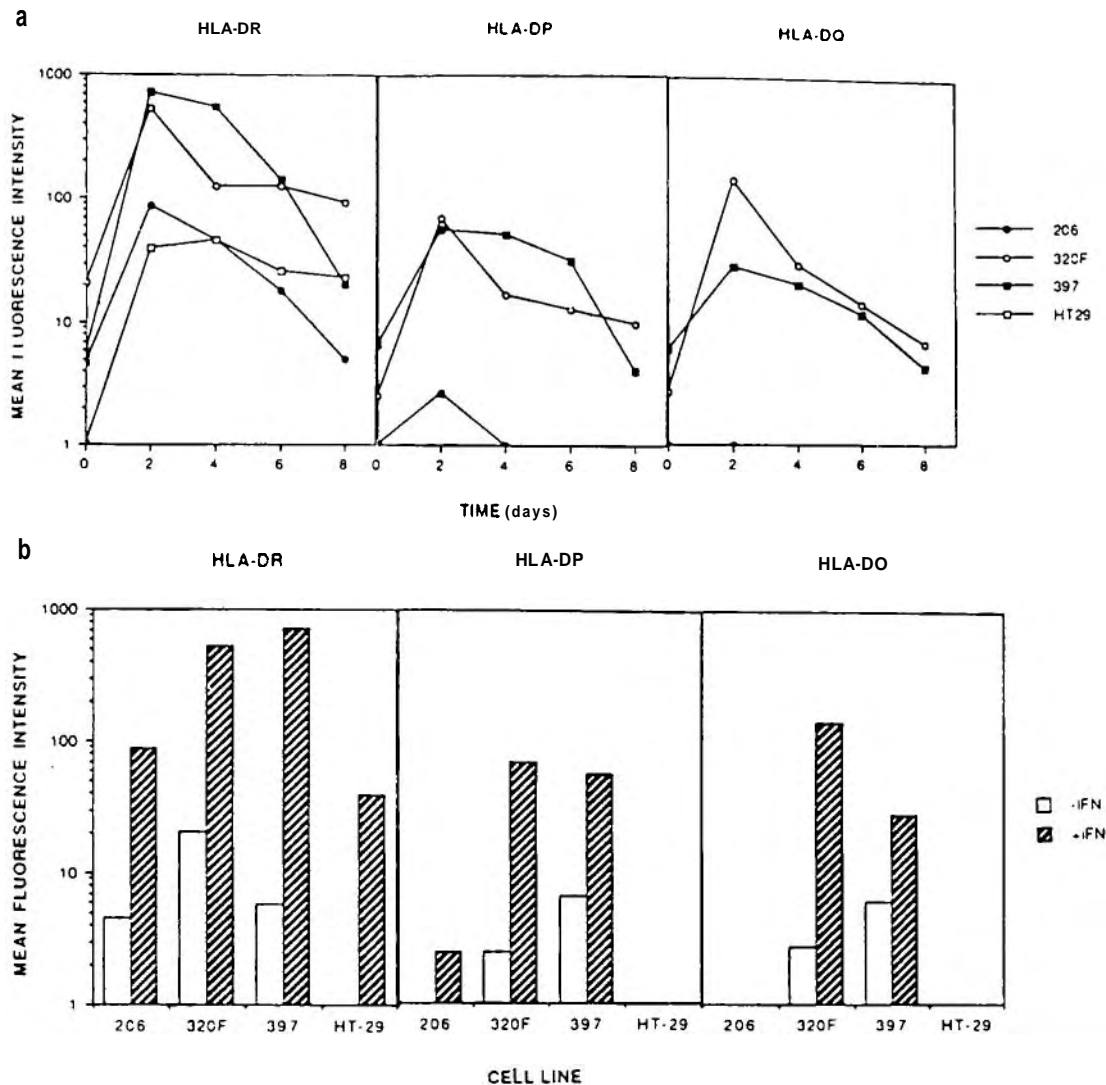


FIG. 3: Levels of expression of class II antigens by four human colonic carcinoma cell lines after treatment with rIFN $\gamma$ . (a) shows the kinetics of expression and (b) summarises the data showing the maximum level of expression. The open bars are untreated cultures and the hatched bars are cultures treated with rIFN $\gamma$ .

In summary, we have found that class I and class II antigens can be upregulated by treatment of cell lines with IFNg. The fact that colonic tumours, unlike these cell lines, express less class I antigen and more class II antigen (as compared to normal colonic tissues) indicates that IFNg is not the only element affecting expression of the MHC antigens by colonic tumours. The difference in the expressions of class I and class II antigens by tumours may be related to intrinsic properties of the tumours, properties which may be quite different from the cell lines used in this study. Furthermore, it appears that IFNg-induced expression of class I antigen occurs by a process different from that of IFNg-induced class II expression.<sup>20</sup> In addition, we can only speculate as to whether IFNg is present in the colon. We have recently found that colonic tumours contain numerous CD4+ T cells.<sup>21</sup> These are almost entirely of the CD45 Ro subset which are the T cells responsible for production of IFNg.<sup>22</sup> However, to draw any conclusion from this is made difficult by the fact that the normal colon, which is class II negative, actually contains a higher density of these CD45 Ro T cells than do tumours, and that colonic carcinomas with poor prognosis have markedly fewer T lymphocytes than tumours with good prognosis, but the former more often expresses class II antigens.<sup>1</sup> No doubt IFNg, in certain circumstances, can induce expression of MHC antigens, but certainly other factors are required for this to occur *in vivo*. One possible explanation for the expression of class II antigens on colonic carcinoma may be the obvious altered biochemistry of the tumour cells themselves.<sup>23</sup> There is currently great interest in trying to unravel the mechanism(s) which control the expression of MHC antigens.<sup>24</sup> Nonetheless, it remains important to establish whether some form of treatment with IFNg can increase the expression of MHC antigens by colonic carcinomas *in vivo* and to investigate whether this will lead to more efficient responses against the tumours.

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