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# The Effect of $\alpha$ and $\gamma$ Interferon on Cell Growth and Histocompatibility Antigen Expression by Human Renal Carcinoma Cells *in vitro*

Rachel Angus, C.M.P. Collins and M.O. Symes

Tumour cells were separated from 19 renal carcinomas and cultured *in vitro*. The effect of interferon (IFN)  $\alpha$  and  $\gamma$  on cell proliferation was measured and compared to the effect of IFN on the expression of class I and class II major histocompatibility complex (MHC) antigens. When tested within the first 14 days of culture, IFN- $\alpha$  inhibited protein synthesis in 12 of 15 and IFN- $\gamma$  in four of nine tumours. Reduction in cell counts was in parallel. In six tumours the culture period was extended and in all six the effect of IFN- $\alpha$  was lost. Exposure to IFN- $\alpha$  induced or enhanced class I antigen expression in eight of 19 tumours and class II expression in two of 19. The analogous figures for IFN- $\gamma$  were five and three tumours. In four of five cases where a comparison could be made there was a correlation between the effects of IFN- $\alpha$  on cell proliferation and class I antigen expression. The efficacy of IFN in the treatment of renal carcinomas may thus, in part, result from inhibition of cell proliferation and enhancement of antigen expression.

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## INTRODUCTION

INTERFERONS HAVE a variety of effects on cells which mitigate against the growth of neoplasms. For example, interferon  $\alpha$  (IFN- $\alpha$ ) inhibits cell proliferation [1], is cytotoxic [2] and amplifies or induces the expression or re-expression of major histocompatibility complex (MHC) antigens [3, 4]. Interferon- $\gamma$  (IFN- $\gamma$ ) activates immune cells, for example, mononuclear phagocytes [5, 6], T-lymphocytes [7] and natural killer cells [8]. It is particularly effective in amplifying or inducing the expression of class II MHC antigen (Ag) [9]. MHC Ag molecules play an important role in the presentation of self or non-self Ag leading to the initiation of an immune response and thus their increased expression may be important in potentiating host reactivity against tumour cells. IFN- $\alpha$  and IFN- $\gamma$  have been used to treat metastatic human renal carcinoma with a response rate of approximately 15% [10–13].

We have recently described a method for the establishment of

human renal carcinoma cells (RCC) in long-term culture [14]. As the identification of a subset of RCC likely to respond to IFN therapy could be clinically useful it seemed germane to study the effect of interferons on the growth and histocompatibility antigen expression by RCC cells cultured from the primary biopsy.

## MATERIALS AND METHODS

### General plan of the experiments

Carcinoma cells from 15 RCC were tested *in vitro* for sensitivity to IFN- $\alpha$  and - $\gamma$ , using the uptake of [ $^{75}\text{Se}$ ]selenomethionine [ $^{75}\text{SeM}$ ], as a measure of protein synthesis, and by cell counting. Cells from each tumour were tested within the first 14 days of culture and in some cases were re-examined after longer periods of *in vitro* growth. At the same time the effect of the interferons on the expression of class I and II MHC Ag was examined. An association was sought between these two effects of interferons.

### Culture of renal carcinoma cells

The methods for separation of carcinoma cells from the RCC [14, 15], the purity of the tumour cell suspensions obtained [15] and their culture [14] have previously been described. A cytospin preparation was made of the cells from the top band on the Nycodenz column for 11 tumours. The cells were stained with

Correspondence to M.O. Symes.

R. Angus and M.O. Symes are at the University Department of Surgery; and C.M.P. Collins is at the University Department of Pathology, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

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May Grunwald Giemsa stain and a differential count involving  $\geq 200$  cells was obtained for 10 tumours: K49, K50, K51, K52, K61, K62, K63, K68, K70 and K73. The mean percentage of tumour cells was  $98.5 \pm 2.5$  (standard deviation, S.D.): for tumour K65 the percentage of tumour cells was 60. The characterisation of the cells from each tumour studied, in the present experiments, was previously detailed by us [14].

### Interferons

IFN- $\alpha$ -N1 (Wellferon) and human natural lymphoblastoid IFN- $\alpha$ , produced by the cell line Namalva, transformed by exposure to the Sendai virus [16], were kindly supplied by Wellcome Biotechnology Ltd. These two types of IFN- $\alpha$  were found to give comparable results in all assay systems. IFN- $\gamma$  (recombinant) was generously donated by Knoll AG.

### Assays for the effect of interferons on cell proliferation

Aliquots of  $10^4$  renal carcinoma cells in RPMI 1640 + 10% v/v newborn calf serum and 1% w/v glutamine (Gibco) were exposed for 24 or 48 h to 100, 500 or 1000 U/ml of IFN- $\alpha$  or - $\gamma$  in round-bottomed polystyrene tubes (Becton Dickinson). Thereafter, the cells were centrifuged (200 g, 5 min) and resuspended with 0.5  $\mu$ Ci [ $^{75}$ SeM] (CIS) in 0.5 ml methionine-free modified Eagle's medium (MEM) (Gibco) for 48 h. The cells were then washed three times in phosphate-buffered saline (PBS) and the incorporated radioactivity measured using a Wilj 2001  $\gamma$  Counter. The percentage inhibition of isotope incorporation in comparison to cells maintained in culture medium alone was determined. All comparisons were based on six individual determinations.

Further aliquots of  $10^4$  carcinoma cells were suspended in 2 ml RPMI (supplemented as above) containing 100, 500 or 1000 U/ml of IFN- $\alpha$  or - $\gamma$  in  $35 \times 10$  mm plastic petri dishes to which the cells adhered. After 4 days the medium was replaced by 2 ml of fresh medium containing the appropriate IFN. At 8 days the medium was replaced by 0.25 ml of PBS containing 0.5 mg/ml trypsin and 0.2 mg/ml EDTA (Gibco). After 10 min at 37°C, 0.25 ml of 0.17% w/v trypan blue was added. After 5 min the number of viable carcinoma cells, recognised by dye exclusion, was estimated by cell counting in a Neubauer Chamber (Hawksley). The percentage inhibition in mean cell numbers, compared to cultures maintained in medium alone was calculated. All comparisons were based on three to six individual determinations.

### Assay for the effect of IFN on expression of MHC Ag by renal carcinoma cells

The methods used have previously been reported [17]. In brief, aliquots of  $10^4$  carcinoma cells in 0.3 ml medium were cultured for 24 h on three-well slides to allow the cells to adhere. The medium was then changed and the cells cultured for 1 or 3 days on three-well slides in medium alone or medium containing 100, 500 or 1000 U IFN- $\alpha$  or - $\gamma$ . The slides were fixed in cold acetone for 10 min. The cells in one well were exposed to monoclonal antibody (MoAb) M736 (Dako). This is derived from clone W6/32 [18] and recognises a monomorphic epitope on the 45 kD polypeptide gene product of the HLA, A, B, C loci (class I MHC Ag). The cells in a second well were exposed to MoAb M704 (Dako) derived from clone DK22. This recognises all gene products from subregions DP, DX and DQ, except DQw1 (class II MHC Ag). The cells in the third well were not exposed to a MoAb and served as a negative control. Reaction with antibody was recognised using the indirect immunoperox-

dase technique, with 3-amino 9-ethylcarbazole as the chromogen.

### Evaluation of staining

All preparations were examined by two independent observers. The number of cells stained were graded as: NIL, 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (>76%). The degree of staining of the individual cells was graded: + (minimal), ++ (moderate) and +++ (marked), with reference to sections of normal human kidney or tonsil stained as positive controls.

### Statistics

For a particular tumour, the individual  $\gamma$  counts for incorporation of [ $^{75}$ SeM] were subject to a one-way analysis of variance and the significance of differences between individual groups was determined using Student's *t*-test based on a pooled estimate of the variance. The significance of differences between mean cell counts was calculated following normalisation of the data, using significance values for the normal distribution.

## RESULTS

### The effect of exposure to IFN- $\alpha$ on protein synthesis

After an initial culture period of <14 days cells from 15 RCC were exposed to increasing concentrations of IFN- $\alpha$  (Wellferon) for 48 h. In six RCC (K49, K50, K61, K67, K70 and K73) protein synthesis, as measured by the uptake of [ $^{75}$ SeM], was inhibited in a dose-dependent fashion (Fig. 1). Protein synthesis was also inhibited in six other tumours (K51, K52, K59, K63, K68 and K69) in a non-dose dependent manner (Fig. 1). In the other three tumours (K60, K62 and K65) no effect of IFN- $\alpha$  was seen. The effect of IFN- $\alpha$  decreased as the culture period increased in the six tumours (K42, K49, K50, K51, K52 and K61) examined (Fig. 2). Exposure to IFN- $\alpha$  for 24 h was without effect on the six tumours examined (K42, K52, K56, K57, K58, K62), data not shown. Tumour K42 showed inhibition of protein synthesis when first exposed to IFN- $\alpha$  for 48 h after 133 days of culture, but the effect was lost as the culture period increased (Fig. 2).

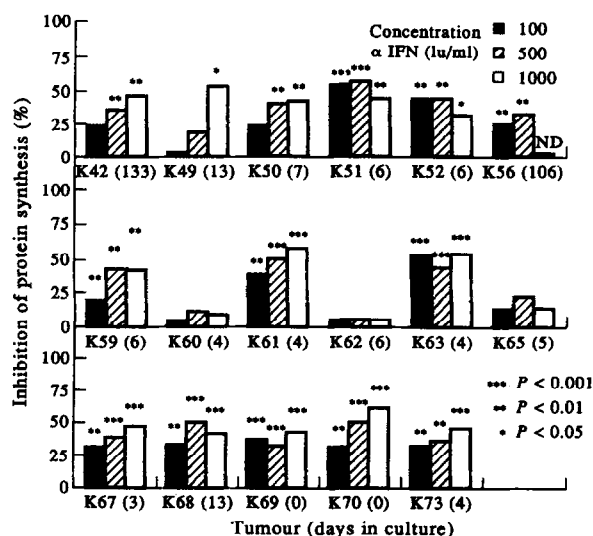


Fig. 1. The effect of 48-h exposure to  $\alpha$ -N1 interferon in inhibiting the subsequent uptake of [ $^{75}$ SeM] over 48 h by renal carcinoma cells *in vitro*. The bars represent the percentage inhibition of isotopic uptake compared to cells maintained in medium without IFN for 48 h prior to the addition of [ $^{75}$ SeM].

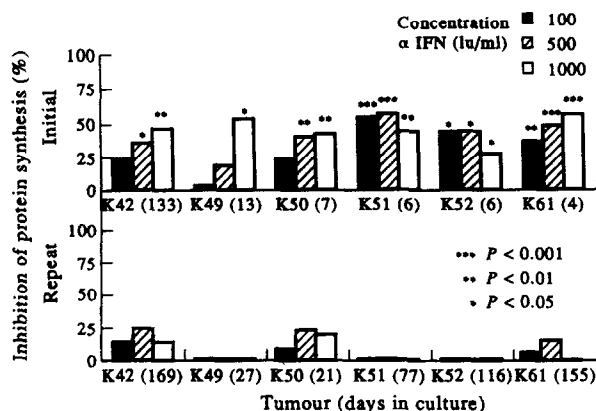


Fig. 2. A comparison of the action of 48-h exposure to  $\alpha$ -N1 interferon in inhibiting the uptake of [ $^{75}$ SeM] by renal carcinoma cells after increasing periods in culture.

#### The effect of IFN- $\alpha$ on cell numbers

Following 8 days exposure to IFN- $\alpha$  (Wellferon), nine recently derived cultures showed a significant reduction in cell numbers, compared to cultures in medium alone (Fig. 3). In four there was a dose response (K42, K57, K68, K73). The other five tumours were inhibited in a dose-independent manner (K56, K58, K61, K63 and K70). In general, the percentage reduction in mean cell count was greater than that seen for inhibition of protein synthesis. However, the significance values for reduction in cell numbers were less due to greater variability in the data.

#### The effect of exposure to IFN- $\gamma$ on protein synthesis

Exposure to IFN- $\gamma$  for 48 h had an inhibitory effect on protein synthesis in four of nine recently derived cultures. In two of these (K63, K70) the level of inhibition was dose dependent,

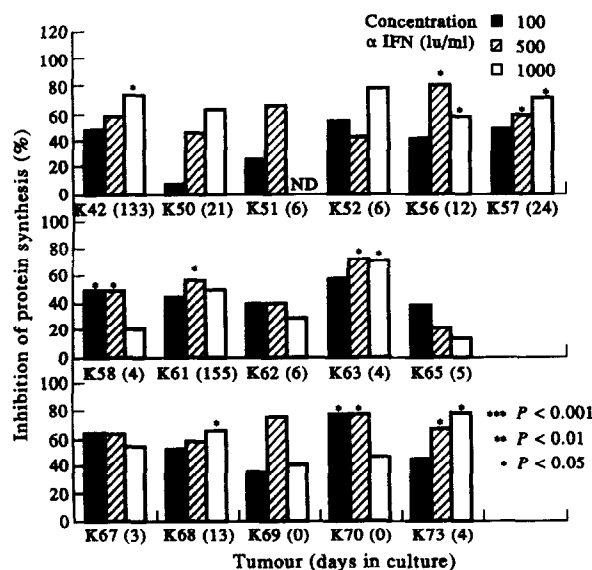


Fig. 3. The effect of 8-day exposure to  $\alpha$ -N1 interferon on cell counts of renal carcinoma cells *in vitro*. The bars represent the percentage reduction in cell numbers compared to that in aliquots of cells not exposed to IFN.

but in the other two tumours (K65, K68) it was not dose related (Fig. 4). No inhibition was seen following 24-h exposure to IFN- $\gamma$ .

#### The effect of IFN- $\gamma$ on cell numbers

Exposure to IFN- $\gamma$  for 8 days led to a significant and dose-dependent reduction in cell numbers in three of eight tumours (K63, K68, K69) and in another tumour (K62), to a non-dose-related decrease (Fig. 4).

#### The relationship between tumour cell purity and the effect of IFN

For 9 of 10 tumour cell suspensions, with a mean tumour cell content from the Nycodenz column of 98.5%, there was a dose-dependent (five cases) and dose-independent (four cases) inhibition of protein synthesis by IFN- $\alpha$ . In two tumours, K62 (100% tumour cells from the column) and K65 (60% tumour cells), IFN- $\alpha$  was without effect (Fig. 1). The effect of IFN- $\gamma$  was determined for six of these tumours. There was no effect in three, dose-dependent inhibition in one and dose-independent inhibition in two, including K65 (Fig. 4). Of the six tumours in which the effect of IFN- $\alpha$  declined as the culture period was increased, the cell purity in five where preparations were obtained from the column was  $97.2 \pm 3.0\%$ .

#### The effect of IFN- $\alpha$ on MHC Ag expression

Four of 19 tumours (K49, K60, K68 and K73) initially showed absence of class I Ag (Table 1). IFN- $\alpha$  produced a dose-dependent induction or increase in class I Ag expression in three of these tumours (K60, K68 and K73) and in five other tumours (K42, K56, K58, K67 and K70) (Table 2). Expression of class II Ag was induced in one tumour (K73) and increased in another (K61).

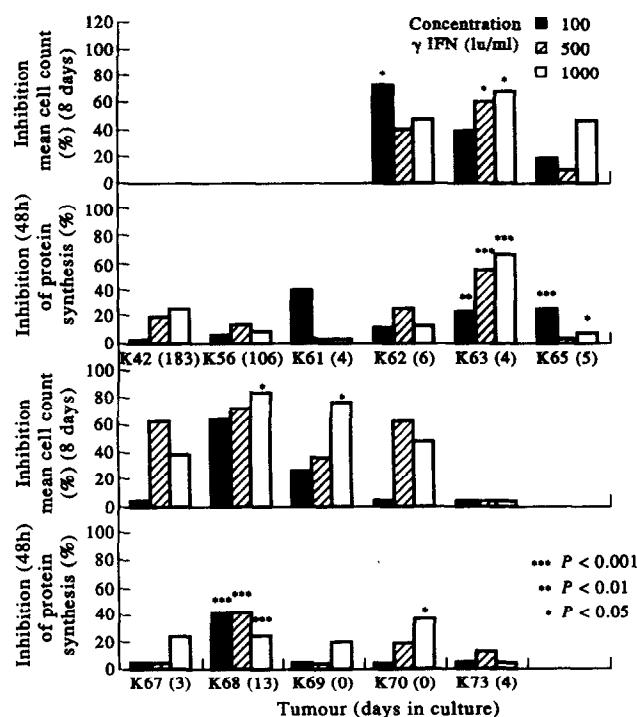


Fig. 4. A comparison of the inhibition in [ $^{75}$ SeM] uptake following 48-h exposure to IFN- $\gamma$  with the direct inhibition of cell numbers following 8-day exposure to IFN- $\gamma$ . The percentage inhibition is calculated with reference to aliquots of cells not exposed to IFN.

Table 1. Expression of class I and class II MHC Ag by renal carcinoma cells in culture

Tumour	Class I MHC Ag	Class II MHC Ag
K42	4 <sup>++</sup> (8) 4 <sup>+</sup> (68) 4 <sup>++</sup> (169) 4 <sup>+</sup> (218)	1 <sup>++</sup> (8) 0(68) 0(169) 0(218)
K49	0(0)	0(0)
K50	4 <sup>+++</sup> (0)	1 <sup>++</sup> (0)
K51	4 <sup>+++</sup> (0)	0(0)
K52	4 <sup>++</sup> +/++(0) 4 <sup>++</sup> (116)	1 <sup>++</sup> (0) 0(116)
K56	4 <sup>+</sup> (12) 2 <sup>+</sup> (106)	0(12) 0(106)
K57	4 <sup>++</sup> +/++(11)	0(11)
K58	4 <sup>+</sup> (4)	1 <sup>++</sup> (4)
K59	4 <sup>++</sup> (6)	1 <sup>++</sup> (6)
K60	0(4) 3 <sup>+</sup> (26)	0(4) 0(26)
K61	4 <sup>+++</sup> (0)	1 <sup>++</sup> (0)
K62	4 <sup>+++</sup> (6)	1 <sup>++</sup> (6)
K63	4 <sup>++</sup> +/++(4)	1 <sup>+++</sup> (4)
K65	4 <sup>++</sup> (5)	0(5)
K67	3 <sup>++</sup> (3)	0(3)
K68	0(13)	1 <sup>++</sup> (13)
K69	4 <sup>++</sup> (0)	1 <sup>++</sup> (0)
K70	3 <sup>++</sup> (0)	1 <sup>++</sup> (0)
K73	1 <sup>++</sup> (4)	0(4)

Cells from each culture were harvested and grown in droplet culture for 1 day prior to estimation of antigen expression. (0): days in culture, (0): cells from the Nycodenz column. No. of cells stained: 4 = 76–100%, 3 = 51–75%, 2 = 26–50%, 1 = 1–25%, 0 = NIL. Degree of staining: +++ = heavy, ++ = moderate, + = light.

#### The effect of IFN- $\gamma$ on MHC Ag expression

IFN- $\gamma$  induced or increased class I Ag expression in five tumours (K42, K49, K60, K68 and K73) and class II Ag expression in three tumours (K42, K60 and K61) (Table 3). Of nine tumours (K42, K49, K56, K58, K60, K67, K68, K70 and K73) which initially showed absence or reduced expression of class I antigen, all except K49 showed upregulation by IFN- $\alpha$  but only five by IFN- $\gamma$ . By contrast, tumour K49 showed upregulation of class I Ag by IFN- $\gamma$  but not by IFN- $\alpha$ .

#### The relationship between expression of class I and class II MHC Ag by tumour cells in culture and the effect of IFN- $\alpha$ and - $\gamma$ in inhibiting protein synthesis

**IFN- $\alpha$ .** There was no relationship between initial expression of MHC Ag by the tumour cells in culture and the effect of IFN- $\alpha$  in inhibiting protein synthesis (Fig. 1, Table 1). Seven tumours were examined after the same culture period for inhibition of protein synthesis and enhancement of MHC class I Ag expression. There was correlation in four tumours (K67, K68, K70 and K73) and no correlation in one (K60). In the other tumours (K59 and K52), expression of class I Ag was already maximal before the addition of IFN.

**IFN- $\gamma$ .** Only one tumour (K68) studied showed a correlation between reduced protein synthesis and increased Ag expression. Otherwise there was no apparent relationship between either initial MHC Ag expression by the tumour cells or its induction by IFN- $\gamma$  and the effect of IFN- $\gamma$  in inhibiting protein synthesis (Fig. 4, Tables 1, 3).

### DISCUSSION

IFN- $\alpha$  is an effective form of therapy for metastatic RCC in approximately 15% of patients [10–13]. The majority of RCC

are, however, unresponsive. IFN- $\alpha$  is both cytotoxic [2] and inhibits cell proliferation [1], in addition to exerting indirect effects via the host immune response, for example, by alteration of MHC Ag expression [3, 4]. Identification of a subset of RCC likely to respond to IFN therapy would be diagnostically useful. This paper has shown that both IFN- $\alpha$  and - $\gamma$  have direct antiproliferative action on recently derived RCC cells. IFN- $\alpha$  was effective against more tumours than IFN- $\gamma$  and sensitivity to IFN was not necessarily dose dependent. Thus, *in vitro* RCC cells can be divided into subsets of those that are sensitive to the effects of IFN and those that are resistant. A study by Nanus *et al.* [19], involving IFN- $\alpha$  treatment of RCC cell lines showed comparable results. Furthermore, they showed that the sensitive RCC lines when grown as tumour xenografts in mice were also markedly inhibited by systemically administered IFN- $\alpha$ . This indicates the relevance of such *in vitro* work in an *in vivo* situation, although it is difficult to define the exact effects of systemically administered IFN.

Cell selection during tumour growth *in vitro* [14] may be similar to that which occurs in the formation of metastases [20]. Thus the decline in the effect of IFN- $\alpha$  on cell proliferation, noted after increasing periods of culture for the cells from all six tumours studied, may be a pointer to the reduced efficacy of this agent in treating patients with metastases arising after prolonged tumour growth.

We have also demonstrated the ability of IFN- $\alpha$  and - $\gamma$  to enhance MHC Ag expression in tumour cells recently derived from RCC. In relation to enhancement of class I Ag expression IFN- $\alpha$  displayed a broader spectrum of activity than IFN- $\gamma$ . However, IFN- $\gamma$  induced class II Ag in only three of 19 tumours in contrast to its usual efficacy [9], but the effect of IFN- $\gamma$  on cultures of normal kidney was not investigated in the present study. In theory, any enhancement in an *in vivo* situation would lead to an increase in both cytotoxic (class I Ag recognition) and helper (class II Ag recognition) T cell involvement in an antitumour immune response. However, MHC Ag have been shown to influence tumour cell growth directly, in addition to their classical role in antitumour immunity. Sunday *et al.* [21] transfected a class I gene into a human neuroendocrine carcinoma cell line COLO 320. They found an increased ability of the tumour cells expressing the H-2L<sup>d</sup> gene to grow as colonies in soft agar and to form metastatic lung colonies in nude mice. Further support for the idea that increased MHC Ag expression may be associated with the neoplastic phenotype comes from the demonstration of increased MHC class I Ag expression on hepatocellular carcinoma cells in comparison to normal hepatocytes [22, 23]; and our demonstration of increased MHC Ag expression in renal carcinoma cells compared to normal renal tubular cells [17]. Several mechanistic pathways have been postulated for these effects. One hypothesis is that an increase in MHC Ag, with its phosphorylated intracellular threonine and serine, could serve as a phosphate donor in tyrosine phosphorylation of growth factor receptors, or otherwise facilitate the signal transduction mechanism triggering DNA synthesis and cell division [21]. Thus, the ability of IFN to increase MHC Ag expression may not be as clinically useful as was originally suggested. However, in a human colon carcinoma cell line HCT-15, MHC class I gene transfection has been shown to inhibit clonogenic growth [24]. This further illustrates the complexity of the signalling pathways controlling growth.

A number of studies have sought a correlation between the effects of cytokines on MHC Ag expression and on the inhibition of cell proliferation. In agreement with Beniers *et al.* [25], who

Table 2. The effect of IFN- $\alpha$  on the expression of class I and class II MHC Ag by the renal carcinoma cells in culture

No.	IFN- $\alpha$ concentration (U/ml)	Class I MHC Ag		Period (days)	IFN- $\alpha$ concentration (U/ml)	Class II MHC Ag		Period (days)
		1 day spot	3 day spot			1 day spot	3 day spot	
K42	Nil	4 <sup>+</sup>	4 <sup>+</sup>	(68)				
	100	4 <sup>+++</sup>	4 <sup>++</sup>					
	500	4 <sup>+++</sup>	4 <sup>++</sup>					
	1000	4 <sup>+++</sup>	4 <sup>++</sup>					
K56	Nil	0	4 <sup>+</sup>	(12)				
	100	0	4 <sup>++</sup>					
	500	2 <sup>+</sup>	4 <sup>+++</sup>					
	1000	4 <sup>+</sup>	4 <sup>+++</sup>					
K58	Nil	4 <sup>+</sup>	4 <sup>+++</sup>	(4)				
	100	4 <sup>+</sup>	4 <sup>+++</sup>					
	500	4 <sup>++</sup>	4 <sup>+++</sup>					
	1000	4 <sup>+++</sup>	4 <sup>+++</sup>					
K60	Nil	0	1 <sup>++</sup>	(4)				
	100	1 <sup>+</sup>	4 <sup>+</sup>					
	500	1 <sup>+</sup>	4 <sup>++</sup>					
	1000	4 <sup>+</sup>	4 <sup>+++</sup>					
	Nil	3 <sup>+</sup>	4 <sup>+</sup>	(26)				
	100	4 <sup>+</sup>	4 <sup>++</sup>					
	500	4 <sup>+</sup>	4 <sup>++</sup>					
	1000	4 <sup>++</sup>	4 <sup>++</sup>					
K61	Nil				Nil	1 <sup>++</sup>	1 <sup>++</sup>	(0)
					100	1 <sup>++</sup>	2 <sup>++</sup>	
					500	1 <sup>++</sup>	2 <sup>++</sup>	
					1000	1 <sup>++</sup>	2 <sup>++</sup>	
K67	Nil	2 <sup>++</sup>	1 <sup>+</sup>	(3)				
	100	4 <sup>++/+++</sup>	3 <sup>++</sup>					
	500	4 <sup>++/+++</sup>	4 <sup>+++</sup>					
	1000	3 <sup>++</sup>	4 <sup>++/+++</sup>					
K68	Nil	—	0	(13)				
	100	—	2 <sup>+++</sup>					
	500	—	4 <sup>++</sup>					
	1000	—	4 <sup>++/+++</sup>					
K70	Nil	3 <sup>++</sup>	4 <sup>++/+++</sup>	(0)				
	100	3 <sup>++</sup>	4 <sup>+</sup>					
	500	4 <sup>++</sup>	4 <sup>++</sup>					
	1000	4 <sup>++</sup>	4 <sup>++</sup>					
K73	Nil	0	1 <sup>++</sup>	(4)	Nil	0	0	(4)
	100	2 <sup>++</sup>	1 <sup>++</sup>		100	1 <sup>++</sup>	0	
	500	0	1 <sup>++</sup>		500	1 <sup>++</sup>	1 <sup>++</sup>	
	1000	2 <sup>++</sup>	2 <sup>++</sup>		1000	TF	1 <sup>++</sup>	

—: not done, TF: technical failure. IFN- $\alpha$  was without effect on the other tumours.

used renal cell carcinoma xenografts, we found no correlation between base level class I or class II Ag expression and sensitivity to cytokine treatment. Beniers *et al.* noted that substantial upregulation of both class I and class II Ag was apparently indicative of a good response to cytokine treatment; and that upregulation of either class I or class II Ag correlated with moderate sensitivity of the tumour cell line. In our studies the majority of tumours showed increases in either class I or class II expression and not in both. To relate changes in MHC Ag expression to a decrease in protein synthesis we need to compare equivalent periods of culture for each tumour. We report a possible correlation between an increase in class I Ag expression

in response to IFN- $\alpha$  and significant inhibition of protein synthesis (dose related and dose independent) in four of five evaluable tumours. The data is insufficient to reach other conclusions, although one of two tumours studied showed inhibition of protein synthesis and an increase in class I MHC Ag in response to IFN- $\gamma$ . In accordance with Beniers, we found that the greatest inhibition of protein synthesis did not necessarily correlate with the maximum rise in class I or class II Ag expression for a given concentration of IFN- $\alpha$ . In contrast, Gastl *et al.* [1] reported a dissociation between antiproliferative activity and induction of HLA-DR Ag expression in response to IFN- $\alpha$  and - $\gamma$ . They studied breast cancer cell lines, i.e. a

Table 3. The effect of IFN- $\gamma$  on the expression of class I and II MHC Ag by renal carcinoma cells in culture

No.	IFN concentration (U/ml)	Class I MHC Ag		Period (days)	IFN concentration (U/ml)	Class II MHC Ag		Period (days)
		1 day spot	3 day spot			1 day spot	3 day spot	
K42	Nil	4 <sup>+</sup>	4 <sup>+</sup>	(68)	Nil	1 <sup>++</sup>	2 <sup>++</sup>	(8)
	100	4 <sup>+++</sup>	4 <sup>+</sup>		100	1 <sup>+</sup>	3 <sup>+</sup>	
	500	4 <sup>+++</sup>	4 <sup>++</sup>		500	1 <sup>++</sup>	4 <sup>++</sup>	
	1000	4 <sup>+++</sup>	4 <sup>++</sup>		1000	1 <sup>++</sup>	4 <sup>++</sup>	
					Nil	0	0	
				(169)	100	0	1 <sup>++</sup>	
					500	0	1 <sup>++</sup>	
					1000	0	1 <sup>++/+++</sup>	
K49	Nil	0	TF	(0)				
	100	0	TF					
	500	4 <sup>+</sup>	TF					
	1000	4 <sup>+</sup>	TF					
K60	Nil	0	1 <sup>++</sup>	(4)	Nil	0	0	(4)
	100	1 <sup>++</sup>	4 <sup>++</sup>		100	0	1 <sup>++</sup>	
	500	1 <sup>+/++</sup>	4 <sup>++</sup>		500	0	1 <sup>++</sup>	
	1000	4 <sup>+</sup>	4 <sup>+++</sup>		1000	0	3 <sup>++</sup>	
	Nil	3 <sup>+</sup>	4 <sup>+</sup>	(26)				
	100	4 <sup>+</sup>	4 <sup>+</sup>					
	500	4 <sup>+/++</sup>	4 <sup>+/++</sup>					
	1000	4 <sup>++</sup>	4 <sup>++</sup>					
K61					Nil	1 <sup>++</sup>	1 <sup>++</sup>	(0)
					100	1 <sup>++</sup>	1 <sup>++</sup>	
					500	1 <sup>++</sup>	3 <sup>++</sup>	
					1000	1 <sup>++</sup>	2 <sup>++</sup>	
K68	Nil	—	0	(13)				
	100	—	1 <sup>++</sup>					
	500	—	1 <sup>++</sup>					
	1000	—	3 <sup>+/++</sup>					
K73	Nil	0	1 <sup>++</sup>	(4)				
	100	1 <sup>++</sup>	1 <sup>++</sup>					
	500	1 <sup>++</sup>	1 <sup>++</sup>					
	1000	1 <sup>++</sup>	1 <sup>++</sup>					

—: not done, TF: technical failure. IFN- $\gamma$  was without effect on the other tumours.

different tumour type and this could account for the discrepancies seen.

In conclusion, IFN- $\alpha$  and - $\gamma$  both inhibit proliferation and increase MHC Ag expression by cells in cultures recently derived from the parent neoplasm. However, the effect on cell proliferation was less pronounced on prolonged tumour growth and may, therefore, be less in evidence when IFN is given in the treatment of metastatic tumours.

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# Radioimmunodetection of Human Small Cell Lung Carcinoma Xenografts in the Nude Rat Using <sup>111</sup>In-labelled Monoclonal Antibody MOC-31

Martinus W.A. de Jonge, Jos G.W. Kosterink, Yu Yan Bin, Jeff W.M. Bulte, Roland A.M. Kengen, Do A. Piers, T. Hauw The and Lou de Leij

The applicability of mouse monoclonal antibody MOC-31 for *in vivo* radioimmunodetection of human small cell lung cancer (SCLC) was investigated in a nude rat xenograft model. MOC-31 is reactive with a 38 kD pan-carcinoma membrane antigen. [<sup>111</sup>In]DTPA-MOC-31 showed good *in vivo* immunolocalisation to xenografted SCLC cells, whereas antigen-related uptake was low in normal rat tissues and in a control antigen-negative, human-derived tumour. Non-antigen-related uptake in the liver could be blocked by pretreatment with irrelevant antibody. It is concluded that MOC-31 can be used for radioimmunodetection of SCLC *in vivo* and may be suitable as a targeting device in patients.

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## INTRODUCTION

MONOCLONAL ANTIBODIES (Mab) provide good opportunities for diagnostic and therapeutic applications in patients [1–3]. Most patients examined so far in radioimmunodetection trials suffered from melanoma, lymphoma, colon, breast or ovarian carcinoma.

Although lung cancer is also an important target for radioimmunodetection or radioimmunotherapy, few studies have been published on this type of cancer [4, 5]. Based on histological and clinical findings, lung cancer can be divided into small cell lung carcinoma (SCLC) and non-SCLC [6]. SCLC is characterised by a high metastatic capacity and a high initial sensitivity to chemo- or radiotherapy. Although good initial responses to chemo- or radiotherapy are obtained in most SCLC patients, almost all patients show therapy-resistant recurrences within 0.5–1 year and as a result survival is very low [7, 8]. To overcome the bad prognosis for SCLC patients, additional treatment modalities are needed. Mabs might be helpful for such a requirement in the future. A number of Mabs directed against SCLC-associated antigens has been developed [7–14]. One of these, MOC-31, which has been clustered as an SCLC-cluster 2 antibody during

Correspondence to M.W.A. de Jonge.

M.W.A. de Jonge, J.W.M. Bulte, T. Hauw The and L. de Leij are at the Department of Clinical Immunology, University of Groningen, Oostersingel 59, 9713 EZ Groningen, The Netherlands; J.G.W. Kosterink is at the Department of Pharmacy; R.A.M. Kengen and D.A. Piers are at the Department of Nuclear Medicine, University Hospital Groningen, The Netherlands; and Y.Y. Bin is at the Department of Biochemistry and Immunology, Institute for Medical Research, China–Japan Friendship Hospital, Beijing, China.

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