

# Anti-interferon- $\gamma$ Antibody Treatment, Growth of Lewis Lung Tumours in Mice and Tumour-associated Cachexia

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C57BL/6N mice bearing Lewis lung tumours were treated with anti-gamma-interferon (IFN- $\gamma$ ) monoclonal antibodies. Early, but not late, treatment inhibited tumour growth, suggesting that endogenous IFN- $\gamma$  promotes initial tumour cell proliferation. Tumour development was associated with failure to gain weight or with progressive weight loss. Anti-IFN- $\gamma$  given early or late counteracted this wasting syndrome, which indicates that IFN- $\gamma$  production subsists during tumour growth and is directly or indirectly responsible for tumour-associated cachexia. Studies of body composition in cachectic mice revealed fat tissue to be particularly affected. Fat loss was enhanced by IFN- $\gamma$  and antagonised by anti-IFN- $\gamma$ . Tumour-bearing mice were also hypersensitive to the lethal effect of endotoxin; anti-IFN- $\gamma$  was unable to mitigate this sensitisation, suggesting that IFN- $\gamma$  does not exert its cachexia-inducing effect through augmentation of the host response to an endogenous endotoxin source. *Eur J Cancer*, Vol. 27, No. 2, pp. 182–187, 1991.

## INTRODUCTION

CYTOKINES occupy a central place in the interaction between tumour and host. On the one hand, they are a means by which the host develops a defence reaction against the tumour. Certain cytokines have cell growth inhibitory (e.g. alpha or beta interferon [IFN]) [1] or cytotoxic effects (e.g. tumour necrosis factor [TNF], lymphotoxin [LT]) [2, 3]; others have activating effects on immune cells, such as mononuclear phagocytes (IFN- $\gamma$ ) or lymphocytes (e.g. interleukin 1 [IL-1]) [4–6], whose activities are necessary to kill cancer cells. On the other hand, cytokines produced by tumour cells or the immune system can modulate tumour growth but may also cause symptoms in the host. Various cytokines, such as transforming growth factor beta and IL-6 (i.e. hybridoma/plasmacytoma growth factor), are growth factors, and have a positive effect on tumour cell proliferation [7, 8]. In addition they induce proteolytic enzymes, such as plasminogen activators [9] and matrix metalloproteases [10], responsible for the degradation of the extracellular matrix, which may promote invasion and metastasis [11]. Furthermore, TNF and IL-1, as attested by their respective alternative names “cachectin” and “catabolin”, are held responsible for cachexia [12–14] associated with chronic infections or cancer.

TNF and IL-1 have also been implicated in acute inflammatory responses associated with exposure to bacterial endotoxins: endotoxin shock and increased sensitivity of tumour-bearing animals to endotoxin [13, 15–17]. However, it has also been shown that, besides IL-1 and TNF, IFN- $\gamma$  is important in the pathogenesis of endotoxin-associated reactions, such as the generalised Shwartzman reaction [18]. We have therefore tested the hypothesis that IFN- $\gamma$  is also involved in cachexia and in increased endotoxin-sensitivity of tumour-bearing mice.

## MATERIALS AND METHODS

**Mice.** Female C57BL/6N mice were originally obtained from Charles River Wiga, Sulzfeld, Germany. All experiments were done in 6–8-week-old, weight-stable mice. Animals had free access to water and standard food whereas tumour-bearing, paired controls were allowed access to the average quantity of food consumed by tumour-bearing mice treated with IFN- $\gamma$  during the previous 24 h. Food intake and body weight were recorded.

**Tumour model.** A line of Lewis lung (LL) carcinoma cells was developed from a transplantable tumour (provided by Dr G. Atassi, Institute Bordet, Brussels) and maintained *in vitro* with EMEM medium (Gibco) supplemented with 10% heat-inactivated fetal calf serum (FCS) (Boehringer Mannheim), 2 mmol/l glutamine and 2.2 g/l sodium bicarbonate. On the day of tumour cell inoculation (day 0),  $10^{5.3}$  tumour cells were suspended in 100  $\mu$ l EMEM supplemented with 2% FCS and injected intramuscularly in the right leg. From day 7, tumour growth could be assessed by measuring the diameter of the tumour with calipers in two perpendicular directions. Volume was calculated with  $4\pi ab^2/3$  ( $a$  = largest radius,  $b$  = smallest radius).

**Body composition and tumour weights.** Animals were killed on day 11, 13 and 15 (Fig. 3) or day 15 (Fig. 7 and Table 2) by cervical dislocation. The tumours were dissected out and weighed wet. Each carcass and dissected tumour were placed in an oven at 80°C until constant weight was reached (dry weight). Carcass fat content was measured [19]. After the extraction (8 h), extracts were dried and the amount of lipids was quantified by weighing.

**Reagents.** Phenol-extracted *S. marcescens* lipopolysaccharide (LPS) was purchased from Sigma (L6136, lot 93F-4019). Monoclonal antibody against MuIFN- $\gamma$  was obtained as ascites fluid from athymic nude mice inoculated with rat  $\times$  mouse hybridoma line F3 (IgG2a) [18]. The antibody was purified by affinity chromatography on a monoclonal anti-rat-kappa chain antibody. Neutralising titre was  $10^{5.5}$  for ascites fluid and  $10^{5.75}$  for pure antibody. The monoclonal F3 antibody had no direct effect on LL tumour cell proliferation *in vitro*. Control ascites fluid was

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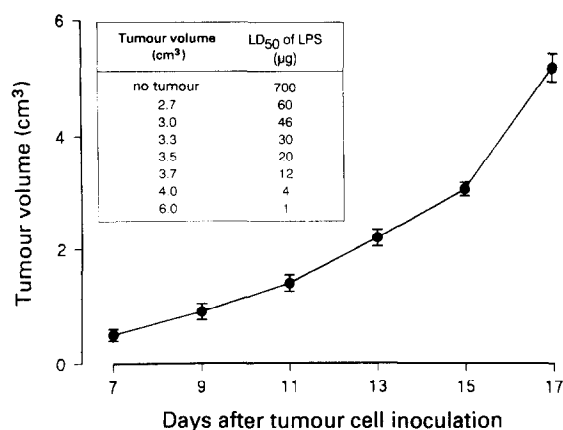


Fig. 1. Tumour volume as a function of time.  $10^{5.3}$  LL tumour cells were injected intramuscularly on day 0. Points represent averages (S.E.) ( $n = 5$ ). Inset: increased sensitivity of LL tumour bearing mice to the lethal effect of LPS in relation to tumour volume.

obtained from mice carrying a rat  $\times$  mouse hybridoma that did not produce antibodies against IFN- $\gamma$ . Irrelevant rat IgG2a was obtained from ascites fluid of a rat plasmacytoma through the courtesy of Dr H. Bazin, University of Louvain Medical School, Brussels. Both antibody and control rat IgG were injected intraperitoneally 0.1 ml, unless otherwise indicated. Murine IFN- $\gamma$  was obtained from the supernatant fluid of DICK cells, a Chinese hamster ovary cell developed in our laboratory [20] that carries and expresses an amplified murine IFN- $\gamma$  cDNA. It was purified by affinity chromatography on the F3 monoclonal antibody to a specific activity of  $10^{7.1}$  U/mg (range  $10^{6.5}$ – $10^{8.0}$ ) [18]. Batches of anti-IFN- $\gamma$  antibodies, control ascites fluid and IFN- $\gamma$  contained less than 2 ng/ml endotoxin (chromogenic Limulus amoebocyte lysate assay, KabiVitrum).

## EXPERIMENTAL DESIGN AND RESULTS

### Effects of LL tumours on body weight and sensitivity to LPS

Intramuscular injection of  $10^{5.3}$  LL tumour cells in C57BL/6N mice caused subcutaneous tumours that were measurable from day 7 onwards (Fig. 1) and which continued to grow to 5–6 cm<sup>3</sup> on day 17 after injection. The tumour-bearing mice failed to gain weight or even lost weight, whereas healthy controls gained about 2 g body weight over the 2-week observation period (see also Fig. 4).

From day 13 after tumour cell inoculation, groups of 4 mice were challenged with different doses of LPS intraperitoneally in 0.1 ml pyrogen-free phosphate-buffered saline (PBS). Deaths were recorded within 48 h and 50% lethal doses (LD<sub>50</sub>) were calculated. To study sensitisation of such mice to the lethal effect of LPS, tumour-bearing mice were grouped according to tumour size and used for LD<sub>50</sub> assays. In healthy controls LD<sub>50</sub> was about 700 µg per mouse. This value was reduced to 20 and 1 µg in mice bearing tumours of 3.5 and 6.0 cm<sup>3</sup>, respectively (Fig. 1, inset). Thus the tumour caused up to a 700 fold increase in sensitivity to LPS.

### Anti-IFN- $\gamma$ antibody and tumour growth

At different times groups of 5 tumour-bearing mice were given anti-IFN- $\gamma$  antibodies or control ascites fluid. In two independent experiments (experiments 1 and 2) in which anti-body treatment was started before tumour cell inoculation,

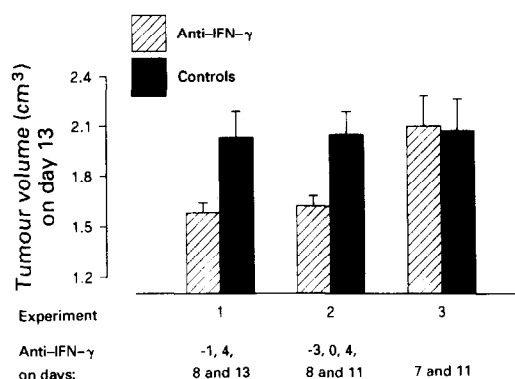


Fig. 2. Inhibition of LL tumour growth in mice by treatment with anti-IFN- $\gamma$  antibody. Anti-IFN- $\gamma$  antibody (F3, ascites fluid, 0.1 ml in experiment 1 and 2, 0.2 ml in experiment 3) was given intraperitoneally on time points indicated (days relative to tumour cell inoculation). Error bars represent S.E.,  $n = 5$  mice per group. ANOVA yielded  $P < 0.05$  for treatment schedules used in experiments 1 and 2.

tumour growth decreased (Fig. 2). In a third experiment, in which antibody treatment was started late (day 7), no effect was seen.

In an additional experiment dry weights were measured at different times after tumour cell inoculation. Six groups of 5 mice each were inoculated with LL tumour cells; three groups were treated with monoclonal antibody against IFN- $\gamma$  and three received control rat IgG (Fig. 3). On days 11, 13 and 15, pairs from antibody-treated and control groups were killed and tumour dry weights were recorded. The decrease in tumour volume in the anti-IFN- $\gamma$  treated group was reflected in a significantly smaller tumour dry weight.

### Effect of anti-IFN- $\gamma$ antibody and IFN- $\gamma$ on body weight and food intake

Groups of 12 mice inoculated with LL tumour cells also received repeated injections of anti-IFN- $\gamma$  antibodies or a control rat IgG (Fig. 4). A group without tumour was included for comparison. As was the case in previous experiments, tumour volumes were significantly smaller in the mice treated with anti-IFN- $\gamma$  antibody (1.986 [0.077] on day 13) than in control treated

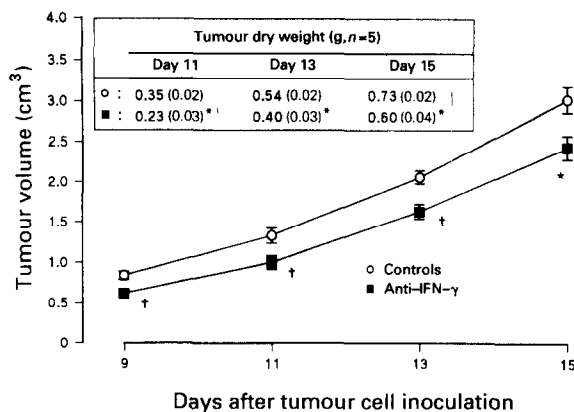


Fig. 3. Effect of anti-IFN- $\gamma$  treatment on tumour volume and tumour dry weight. Purified anti-IFN- $\gamma$  antibody or control rat IgG (0.1 ml) was given intraperitoneally on days -2, 0, 7 and 11. Points represent averages (S.E.) for  $n = 15$  (day 9 and 11),  $n = 10$  (day 13) and  $n = 5$  (day 15) mice/group. Tumour dry weights (inset) represent averages (S.E.) for  $n = 5$  mice/group.

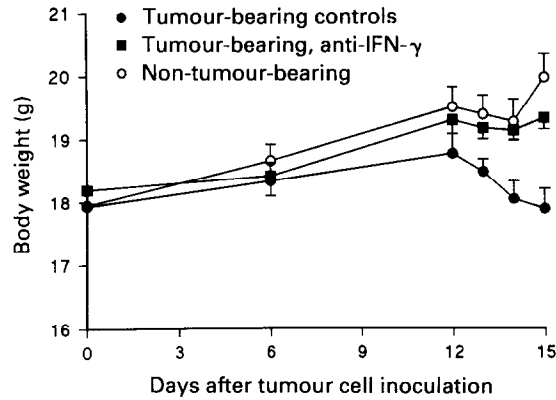


Fig. 4. Effect of anti-IFN- $\gamma$  treatment on body weight of tumour-bearing mice during a period of 15 days following tumour cell inoculation. Tumour cell challenge:  $10^{5.3}$  cells intramuscularly. Purified anti-IFN- $\gamma$  antibody or irrelevant rat IgG (0.1 ml) was given intraperitoneally on days -1, 2, 6 and 10 relative to day of tumour cell inoculation. The points represent means (S.E.) for 12 mice/group.  $P < 0.05$  (day 13),  $P < 0.005$  (day 14) and  $P < 0.001$  (day 15) for anti-IFN- $\gamma$  treatment vs. tumour-bearing control (ANOVA).

mice ( $2.373 [0.130] \text{ cm}^3$ ,  $P < 0.05$ ). In all groups body weight increased till about day 12. From then on, tumour-bearing mice that were not treated with monoclonal antibody against IFN- $\gamma$  progressively lost weight. In contrast, the body weight of non-tumour-bearing mice or tumour-bearing mice treated with anti-IFN- $\gamma$  antibody remained stable. Changes in body weight in this experiment as well as in a second experiment are plotted in Fig. 5A. In both experiments, tumour-associated weight loss was significantly reduced by antibody treatment. Furthermore, body weight changes in tumour-bearing mice treated with anti-IFN- $\gamma$  antibodies ranged within the values for untreated non-tumour-bearing mice.

To control confounding by tumour volume, the data from

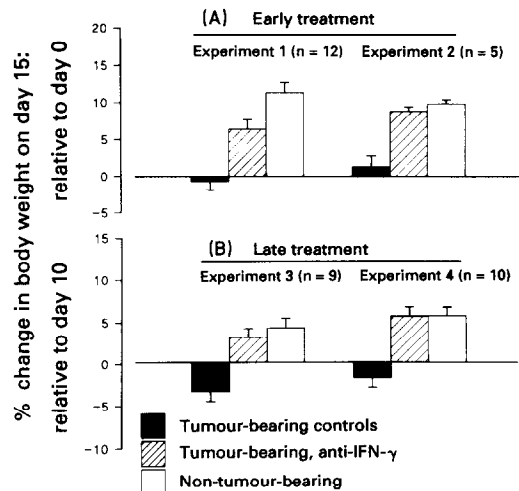


Fig. 5. Diminished weight loss in LL tumour-bearing mice treated with anti-IFN- $\gamma$  antibodies in an early and late time schedule. Panel A: pure anti-IFN- $\gamma$  antibodies (F3, 0.1 ml) or control rIgG was given intraperitoneally on days -1, 2, 6 and 10 (experiment 1) and days -3, 0, 4, 8 and 11 (experiment 2) relative to the day of tumour cell inoculation. Panel B: anti-IFN- $\gamma$  antibody (F3, 0.2 ml) was given intraperitoneally on days 10 and 13 relative to the day of tumour cell inoculation.  $P < 0.01$  (experiments 2, 3);  $P < 0.005$  (experiment 1) and  $P < 0.002$  (experiment 4) for anti-IFN- $\gamma$  treatment vs. tumour-bearing control (ANOVA).

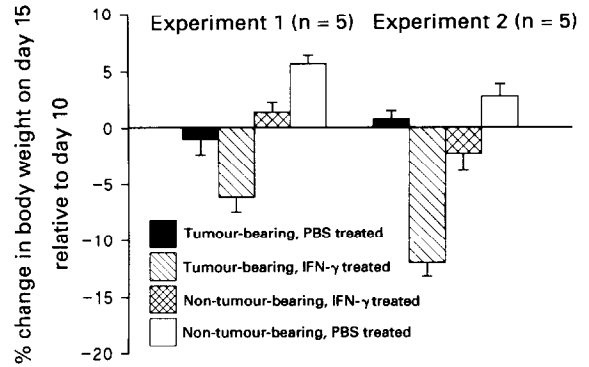


Fig. 6. Enhanced weight loss in LL tumour-bearing mice treated with IFN- $\gamma$ . Treatment schedules: IFN- $\gamma$  ( $10^{5.1}$  U) given intraperitoneally on days 10 and 11 (experiment 1) and days 10, 11, 12 and 13 (experiment 2) relative to the day of tumour cell inoculation.  $P < 0.05$  (experiment 1),  $P < 0.0005$  (experiment 2) for tumour-bearing mice PBS-treated vs. IFN- $\gamma$  treated; and  $P < 0.01$  (experiment 1),  $P < 0.005$  (experiment 2) for IFN- $\gamma$  treatment (tumour-bearing vs. non-tumour-bearing).  $P < 0.05$  (experiments 1 and 2) for non-tumour-bearing mice, PBS-treated vs. IFN- $\gamma$ -treated (ANOVA).

experiment 1 (Fig. 5A) were also analysed by comparing the percentage change in body weight in pairs of mice taken from the control and the antibody treated group and matched for tumour volume on day 10 (i.e. the day when antibody treatment was discontinued). ANOVA with two criteria of classification (treatment group and matched pair) and one entry per box yielded a  $P < 0.01$  for treatment effect.

In the experiments shown in Fig. 5B, tumour-bearing mice were given injections of anti-IFN- $\gamma$  antibody or control rat IgG as late as days 10 and 13: the antibody did not affect tumour size (see also Fig. 2). Nevertheless, as can be seen in Fig. 5B, cachexia was significantly antagonised.

Figure 6 shows the results of two experiments in which the effect of exogenous IFN- $\gamma$  on body weight changes in tumour-bearing mice was studied. Tumour-bearing and control mice received intraperitoneal injections of IFN- $\gamma$  on days 10 and 11 (experiment 1) and on days 10, 11, 12 and 13 (experiment 2). IFN- $\gamma$  had a negative influence on body weight, both in tumour-bearing and control mice. In non-tumour-bearing mice, treatment with IFN- $\gamma$  resulted in a lesser weight gain (experiment 1) or even in weight loss (experiment 2), compared with significant weight gains in controls. Mice with tumours and not treated with IFN- $\gamma$  failed to gain weight; treatment of such mice with IFN- $\gamma$  resulted in significant weight loss.

We also measured daily food intake in the tumour-bearing and control mice of experiment 3 (Fig. 5) and of experiment 1 (Fig. 6). Tumour-bearing mice which were not treated with IFN- $\gamma$  had only a minimal degree of anorexia, which was significant at the late stages of the experiment (from day 13 onwards) (Table 1). Treatment with anti-IFN- $\gamma$  antibody had a slight mitigating effect (not significant) on this anorexia. Treatment with IFN- $\gamma$  had no effect on food intake in non-tumour-bearing mice. In contrast, such treatment caused anorexia in tumour-bearing animals immediately after IFN- $\gamma$  injection.

#### Effect of IFN- $\gamma$ and anti-IFN- $\gamma$ treatment on body composition

Since the anorectic effect of IFN- $\gamma$  might have been responsible for the cachexia, we did an additional experiment in which control groups were pair-fed. Body weight changes of IFN- $\gamma$

Table 1. Food intake in LL tumour-bearing mice treated with IFN- $\gamma$  or anti-IFN- $\gamma$  antibody

Treatment*	Daily food intake (g per mouse) on days:			
	0-10	10-13	13-14	14-15
Experiment 3 ( <i>n</i> = 3)				
Tumour-bearing				
Anti-IFN- $\gamma$	2.9 (0.1)	2.5 (0.2)	2.0 (0.2)	2.1 (0.2)
Control IgG	2.9 (0.2)	2.4 (0.2)	1.6 (0.2)†	1.3 (0.3)†
Non-tumour-bearing				
PBS	3.5 (0.3)	3.1 (0.3)	3.5 (0.1)	3.2 (0.4)
Experiment 1 ( <i>n</i> = 5)				
Tumour-bearing				
IFN- $\gamma$	3.9 (0.3)	2.7 (0.4)‡	2.8 (0.3)‡	2.8 (0.1)‡
PBS	4.0 (0.2)	3.7 (0.4)	3.7 (0.4)	3.2 (0.2)†
Non-tumour-bearing				
IFN- $\gamma$	4.4 (0.3)	4.0 (0.2)	6.0 (0.7)	5.3 (0.5)
PBS	4.8 (0.2)	4.3 (0.2)	4.5 (0.2)	4.7 (0.3)

\*See legend to Figs 5 (experiment 3) and 6 (experiment 1).

†*P* < 0.05 for comparison with non-tumour-bearing controls.

‡*P* < 0.05 (days 10-13) and *P* < 0.005 (days 13-14 14-15) for comparison with non-tumour-bearing IFN- $\gamma$  treated mice.

treated (Fig. 7A) and anti-IFN- $\gamma$  treated mice (Fig. 7B) were followed for 15 days. To determine the body component most affected by IFN- $\gamma$  or anti-IFN- $\gamma$  antibody treatment, body composition at the end of the experiment was also studied (Table 2). As was the case in previous experiments, IFN- $\gamma$  or anti-IFN- $\gamma$  administration (in a late treatment schedule) did not affect tumour growth, since no differences were found in tumour wet or dry weights between treated and untreated groups. In addition, IFN- $\gamma$  caused a significantly higher body weight loss in tumour-bearing mice than in pair-fed controls or non-tumour-

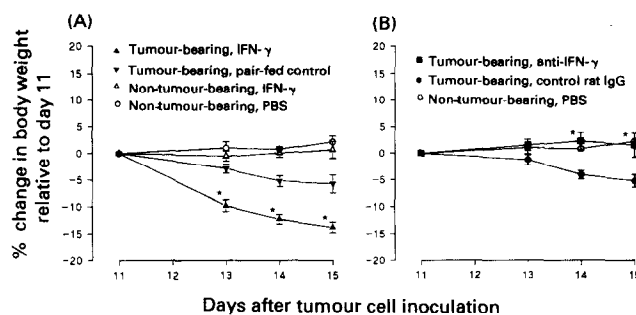


Fig. 7. Body weight changes of tumour-bearing mice during a period of 5 days following IFN- $\gamma$  (panel A) and anti-IFN- $\gamma$  treatment (panel B). Purified anti-IFN- $\gamma$  antibody or control rat IgG (0.1 ml) and IFN- $\gamma$  ( $10^{5.0}$  U/ml) was given on days 11 and 13 relative to the day of tumour cell inoculation. The points represent mean (S.E.) for 5 mice/group. Panel A: *P* < 0.005 for comparison with tumour-bearing, pair-fed mice and *P* < 0.0005 for comparison with non-tumour-bearing, pair-fed mice and *P* < 0.005 for comparison with non-tumour-bearing, IFN- $\gamma$  treated mice; Panel B: *P* < 0.05 for comparison with tumour-bearing, control rat IgG-treated mice (ANOVA).

bearing animals; anti-IFN- $\gamma$  treatment inhibited the spontaneous body weight loss observed in tumour-bearing mice. The weight reduction in untreated tumour-bearing control mice was seen in carcass dry weight and fat content compared with non-tumour-bearing controls (Table 2). This decrease in carcass and fat tissue was even more dramatic in tumour-bearing mice treated with IFN- $\gamma$ . In contrast, anti-IFN- $\gamma$  treatment inhibited the fat loss significantly, whereas the differences in dry carcass weight changes were not significant.

#### Anti-IFN- $\gamma$ antibody and sensitivity to LPS

Table 3 shows three experiments in which LPS induced mortality was compared between groups of tumour-bearing mice that had received anti-IFN- $\gamma$  antibody or control fluid. Since sensitivity to LPS varies with tumour volume (see above), the dose of LPS was adjusted in each group to one estimated LD<sub>50</sub> for mice receiving control fluid. In the first experiment treatment was started early (day -1 relative to tumour cell inoculation).

Table 2. Effect of treatment with IFN- $\gamma$  or anti-IFN- $\gamma$  antibody on body composition of tumour-bearing mice and pair-fed untreated controls

Treatment*	Initial body weight (g)	Final body weight (g)	Non-tumour carcass		Tumour	
			Dry weight (g)	Fat content (g)	Wet weight (g)	Dry weight (g)
Tumour bearing						
IFN- $\gamma$	19.5 (0.4)	17.2 (0.3)†‡	2.81 (0.15)†‡	0.27 (0.02)†‡	3.38 (0.31)	0.64 (0.06)
Anti-IFN- $\gamma$	19.3 (0.3)	20.2 (0.3)§	4.03 (0.13)	0.68 (0.02)§	3.85 (0.36)	0.77 (0.03)
Control IgG	19.6 (0.3)	19.0 (0.3)	3.56 (0.23)	0.49 (0.04)	3.96 (0.28)	0.76 (0.04)
Pair-fed control	19.5 (0.3)	18.7 (0.3)	3.51 (0.21)	0.44 (0.02)	3.70 (0.21)	0.71 (0.04)
Non-tumour-bearing						
IFN- $\gamma$	19.9 (0.4)	20.5 (0.3)	5.28 (0.07)	1.25 (0.02)	—	—
PBS	19.1 (0.2)	20.2 (0.3)	5.50 (0.19)	1.34 (0.07)	—	—

\*See legend to Fig. 7. All animals (5 per group) were killed on day 15 after tumour cell inoculation.

†*P* < 0.05 for comparison with tumour-bearing pair-fed control; ‡*P* < 0.001 for comparison with non-tumour bearing, IFN- $\gamma$  treated mice;

§*P* < 0.05 for comparison with tumour-bearing, control IgG treated mice; and ||*P* < 0.01 for comparison with non-tumour-bearing, PBS treated mice.

Table 3. Effects of anti-IFN- $\gamma$  on the sensitivity of LL tumour-bearing mice to LPS

Experiment	Anti-IFN- $\gamma$ *	LPS ( $\mu$ g)	Average tumour volume (cm <sup>3</sup> )	Mortality
1	+	60	2.9 (0.2)	0/5
	-		3.2 (0.1)	3/5
2	+	30	4.5 (0.1)	4/5
	-		4.5 (0.1)	4/5
3	+	0.5	6.0 (0.2)	2/4
	-		6.1 (0.1)	1/4

\*Days -1, 4, 8 and 13 (experiment 1); days 13, 14, 15 and 16 (experiments 2 and 3).  
†Intraperitoneally on days 15, 16 or 17 for experiments 1, 2 and 3, respectively.  
‡On day of LPS challenge.

There were no deaths in the antibody treated group, whereas in the controls mortality was as expected for the LD<sub>50</sub>. However, since early antibody treatment reduces tumour growth, this reduced mortality might have been accounted for by the lesser tumour load. Therefore, in two subsequent experiments, the antibody treatment was started at a later time (day +13) when it had less or no influence on tumour growth. In these experiments (2 and 3) mortality was not different between antibody treated and untreated mice.

DISCUSSION

Treatment of mice with anti-IFN- $\gamma$  antibody had profound effects on the development of LL tumours and on the accompanying generalised pathology. We interpret this to mean that IFN- $\gamma$  might be produced endogenously in mice bearing LL tumours and that this IFN plays an important role in the evolution of the tumour itself as well as in pathological host reactions associated with the tumour.

Contrary to the expectation that IFN- $\gamma$  would have anti-tumour effects [21, 22] we found that LL tumour growth was antagonised by treatment of the mice with anti-IFN- $\gamma$  antibodies. Several mechanisms may be proposed to explain this effect. Although no IFN- $\gamma$  or TNF- $\alpha$  could be detected in the circulation of the animals (data not shown), IFN- $\gamma$  produced locally by paraesional or tumour-infiltrating lymphocytes might act as a direct growth factor for the LL cells. Reports on the effects of IFN- $\gamma$  on cell growth are equivocal: growth inhibitory [22, 23] as well as growth stimulatory effects [24, 25] have been described. Alternatively, IFN- $\gamma$  may stimulate production of factors, cytokines or other, that act as growth stimulators for LL cells or modulate receptor mediated responses of tumour cells to such activities. IFN- $\gamma$  can activate mononuclear phagocytes as well as endothelial cells [4, 5], and these cells in turn are a rich source of cytokines with growth promoting potential. It is conceivable that inflammation in and around a tumour can sometimes favour rather than antagonise tumour outgrowth. Our data highlight IFN- $\gamma$  as one the factors that may mediate such a mutualism between tumour and inflammatory response. Antibody treatment failed to affect tumour progression if started when the tumours were first measurable. A possible explanation for this failure may be that during nodule development the cells become inaccessible to the trophic effect of the surrounding inflammatory focus. Fully developed LL tumours were indeed invariably found to be encapsulated.

We also demonstrated that weight loss in mice bearing LL tumours was antagonised by treatment with anti-IFN- $\gamma$  antibody or enhanced by exogenous IFN- $\gamma$ , and that these effects are largely or completely independent from the effect on tumour size. Wasting is common in many cancer patients as well as in experimental tumour-bearing animals. There is good evidence that TNF is implicated in the pathogenesis of this cachexia [12-14].

It has often been reported that the presence of a tumour sensitises the host to the toxic effects of bacterial toxins, especially endotoxin from gram-negative bacteria [16, 17]. Our study showed this to be the case for the LL tumour system we used. Since the reactivity of mononuclear phagocytes and endothelial cells to endotoxin is regulated by IFN- $\gamma$  [4, 26], it was of particular interest to see whether the *in vivo* sensitivity of mice to endotoxin would also be controlled by IFN- $\gamma$ . Experiments in which we tried to avoid sensitisation of tumour-bearing mice towards endotoxin, by treating the mice with anti-IFN- $\gamma$  antibody, failed to support this concept. Therefore it seems unlikely that generalised wasting in the LL tumour system is due to sensitisation of the mice to endotoxins.

Several cytokines, including IFN- $\gamma$  [27], affect lipogenesis and/or lipolysis in cells *in vitro*. In our cachexia model the effect of anti-IFN- $\gamma$  antibody was at least partly due to effects on fat storage.

The observation that IFN- $\gamma$  promotes tumour-associated cachexia constitutes a caveat against IFN- $\gamma$  therapy for cancer. However, the fact that anti-IFN- $\gamma$  antibody treatment counteracts this cachexia opens new perspectives for better treatment of cancer patients.

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# Human Papillomavirus DNA as a Possible Index of Invasiveness in Female Genital Tract Carcinomas

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Paraffin-embedded tumour sections were used for the polymerase chain reaction (PCR) with three primer sets that amplify specific regions of human papillomavirus (HPV) types 11, 16 and 18. The positive samples were confirmed by hybridisation of the amplified sequences with the specific HPV probes. In all screened metastases the same viral sequences were found as in the primary tumour. HPV 16 was the most frequently detected virus in genital tract tumours. In a metastatic ovary carcinoma with unknown primary site HPV 16 DNA was observed. Moreover, pelvic lymph nodes with no microscopic evidence of metastases contained HPV DNA of the same subtype as the primary tumour. Thus, the HPV DNA detected by PCR is a useful indicator of neoplastic cells in the earlier stages of invasiveness. The finding of specific HPVs in the metastatic lesions could also provide information about the location of the primary neoplasia.

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## INTRODUCTION

HUMAN PAPILLOMARVIRUSES (HPV) have been associated with tumours of different sites. More than 60 HPV types have been defined but only a few types, mostly 16, 18 and 33, have been detected in squamous carcinoma from anogenital or oropharyngeal sites, whereas other types, such as 6 and 11 have been found in benign lesions [1–3]. The data on the presence of HPV in

tumours have been obtained by Southern or dot-blot techniques and *in situ* DNA hybridisation. The first two techniques have provided evidences for an HPV role in premalignant and malignant lesions [4]. *In situ* hybridisation has revealed, in particular, the correlation between cytological morphology and the presence of HPV genome, and it has also facilitated the retrospective studies of stored samples [5–7]. All of these methods, however, have several limitations due to the low sensitivity of *in situ* techniques in high-grade lesions and to the difficulties of correlating the histological findings with Southern or dot-blot results.

The polymerase chain reaction (PCR) [8] has enhanced the detection of HPV DNA and RNA in clinical specimens and has improved the diagnostic possibilities. To evaluate the presence

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