

## Letter to the Editor

Correspondence re: E. Gorelik *et al.*, Effect of Ultraviolet Irradiation on MCA102 Tumor Cell Immunogenicity and Sensitivity to Tumor Necrosis Factor. *Cancer Res.*, 51: 1521-1528, 1991.

I am writing this letter with regard to a recent paper in *Cancer Research* (1). The authors, Gorelik *et al.*, elegantly demonstrated that MCA102 tumor cells acquire immunogenicity after UV irradiation. These tumor cells are rejected when transplanted in syngeneic hosts and Lyt-2.2 cells appear to be responsible for their rejection. However, the authors state that increased tumorigenicity induced by UV light was not associated with the appearance of class I H-2 antigens. They also indicate that (a) untreated MCA102 tumor cells do not express class I H-2 antigens, and (b) these antigens on normal tumor cells are not regulated by IFN- $\gamma$ .<sup>1</sup>

In contrast to these observations, I have found that MCA102 tumor cells express significant level of MHC class I antigen on their cell surface by utilizing anticlass I antibody specific for H-2D<sup>b</sup> (clone B22-249.R1, Bioproducts for Science, Inc., Indianapolis, IN). This monoclonal antibody was specific for H-2D<sup>b</sup> antigen and generated by immunizing BALB.K mice with C57BL/6 lymphocytes and then fused to NS-1 (2). Single cell suspensions of MCA102 tumor cells were obtained by triple enzyme (hyaluronidase, DNase, and collagenase) digestion of s.c. grown tumor (obtained from Surgery Branch, National Cancer Institute) (3). For my study, these tumors were used between third and seventh passage *in vivo* or *in vitro*. As seen in Fig. 1, MCA102 tumor cells express a significant level of MHC class I H-2D<sup>b</sup> antigen. For example, in the experiment shown, a mean channel number of 155 was observed when stained with anti-H-2D<sup>b</sup> antibody compared to mean channel number of 91 with secondary antibody alone. Up-regulation of MHC class I H-2D<sup>b</sup> antigen expression occurred when tumor cells were incubated with 2000 units/ml IFN- $\alpha$  A/D (specific activity,  $7.9 \times 10^7$  units/mg protein kindly provided by Hoffmann La Roche, Nutley, NJ) or 100 units/ml of r-murine IFN- $\gamma$  (specific activity,  $0.9 \times 10^7$  units/mg protein, kindly provided by Genentech Inc., South San Francisco, CA). This up-regulation occurred after a 72-h incubation with cytokines, but not after a 24-h incubation. Gorelik *et al.* had incubated their tumor cells with IFN- $\gamma$  for only 24 h. Weber and Rosenberg (4) were able to show that MCA102 tumor cells express class I H-2D<sup>b</sup> antigen by utilizing antibodies similar to those used in the Gorelik *et al.* experiment. This expression was up-regulated by 48-h incubation with IFN- $\alpha$  or IFN- $\gamma$ . Also, in this study, both IFNs were able to up-regulate class I expression *in vivo*.

Thus, my data utilizing a monoclonal antibody against H-2D<sup>b</sup> antigen derived from a different clone confirm Weber and Rosenberg's findings. My data demonstrate that MCA102 tumor cells express class I antigen which is up-regulated by IFN- $\alpha$  and IFN- $\gamma$ . These data may have an important bearing on the interpretation of the observations obtained by Gorelik *et al.*

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<sup>1</sup> The abbreviations used are: IFN- $\gamma$ ,  $\gamma$ -interferon; IFN- $\alpha$ ,  $\alpha$ -interferon; MHC, major histocompatibility complex.

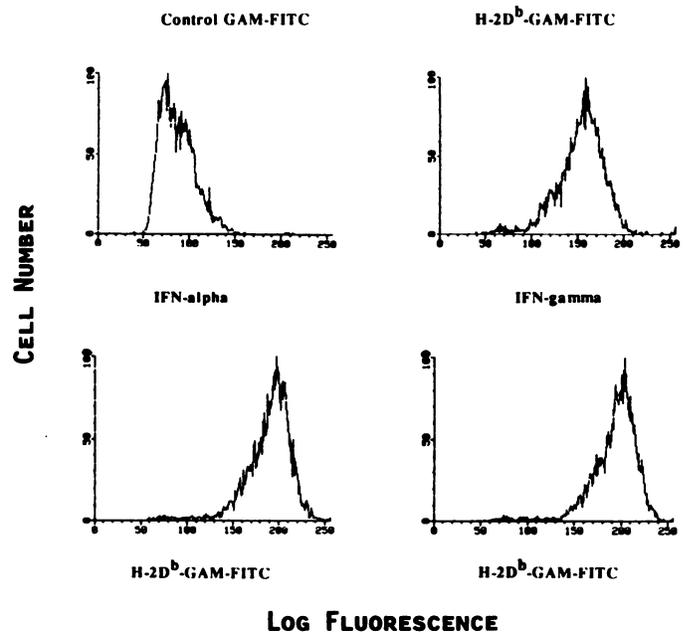


Fig. 1. Flow cytometric analysis of MCA102 tumor cells. Cells were cultured with IFN- $\alpha$  (2000 units/ml) or IFN- $\gamma$  (100 units/ml) for 72 h. Cells were washed and incubated with murine anti-MHC class I H-2D<sup>b</sup> monoclonal antibody. These cells were then stained with fluorescein isothiocyanate (FITC)-labeled second antibody (goat anti-mouse monoclonal antibody). In each frame  $1 \times 10^4$  cells were analyzed by FACScan equipment.

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