Inducibility of Class II Major Histocompatibility Complex Antigens by Interferon γ Is Associated with Reduced Tumorigenicity in C3H Mouse Fibroblasts Transformed by v-Ki-ras

By W. J. Bateman, R. Fiera,* N. Matthews,* and A. G. Morris

Summary

Paired lines of C3H mouse fibroblasts transformed with murine sarcoma virus (Kirsten strain) were prepared that express high or low levels of class II major histocompatibility complex antigen after treatment with interferon γ (IFN-γ). Here, we described a comparison of the tumorigenicity of these lines in euthymic syngeneic and thymus-deficient nu/nu mice and in mice depleted of IFN-γ. The class II-inducible cells are clearly less tumorigenic than the noninducible cells in syngeneic mice, but of similar tumorigenicity in nu/nu mice and in mice treated with antibodies to deplete IFN-γ. We propose that in this system, IFN-γ induction of class II antigens on the tumor cell surface operates to limit tumor growth; ras expression, which inhibits induction of class II antigens, prevents this and so allows tumor growth.

Materials and Methods

Cell Lines. The C3H 10T1/2 fibroblast line was obtained from the American Type Culture Collection (Rockville, MD). The C3H 201 cell line (12) was derived from C3H 10T1/2 by infection with a helper virus-free preparation of the Kirsten strain of murine sarcoma virus (MSV)† prepared by transfection of the β-2 helper cell

† Abbreviation used in this paper: MSV, murine sarcoma virus.
line (20) by a DNA clone of MSV (21). Class II-inducible variants of the transformed cells were prepared as previously described (16) by several cycles of IFN-γ treatment, staining for class II antigens, and sorting low and high expressing cells. Lines so obtained were designated 369L and 369R, respectively (L indicates left sorted and R indicates right sorted). These lines were maintained in MEM (Gibco Laboratories, Paisley, Scotland) supplemented with 10% FCS (Flow Laboratories, Irvine, Scotland) at 37°C in a 95% air 5% CO₂ atmosphere.

**Animals and Tumor Assays.** 6-8-wk-old C3H/He mice from our own animal facility were injected subcutaneously with graded doses of cells in a volume of 0.1 ml of culture medium; MF1 nu/nu mice obtained from OLAC Ltd., Bicester, England, were similarly injected with 300 cells. Tumors, which usually appeared within 7-14 d in euthymic mice and 21-35 d in nu/nu mice, were scored visually and by palpation.

**In Vivo Depletion of IFN-γ with mAbs.** The rat mAb R4-6A2 (22) was purified from ascitic fluid using protein A-Sepharose (Pharmacia Ltd., Milton Keynes, UK) in high salt buffer. C3H/He female mice (6-8 wk old) were injected intravenously with antibody 1 wk before inoculation subcutaneously with 369R or 369L cells, and then given antibody intraperitoneally every 7 d until termination of the experiment. In initial experiments, the amount of mAb given per injection varied from 0 to 200 µg. All amounts >3 µg were found to be equally effective in increasing tumor growth, and a standard dose of 30 µg per injection was subsequently used. Control mice received an equivalent amount of rat immunoglobulin (Sigma Chemical Co., Poole, UK) or no injection. There was no difference observed between these two control groups.

**Results and Discussion**

**Characteristics of Sorted Lines.** The MHC phenotypes of the sorted lines are described elsewhere (16). In brief, neither of the lines expresses class II antigen in the absence of IFN-γ, but after treatment with IFN-γ, the right sorted line 369R expresses about the same as do IFN-γ-induced normal C3H 10T1/2 fibroblasts; the left sorted line 369L is weakly inducible for class II. C3H10T1/2 and 369L or -R express little class I antigen in the absence of IFN-γ, although all lines express very large amounts of class I antigen after IFN-γ treatment (see Fig. 1).

**Tumorigenicity in Immunocompetent Syngeneic Mice.** As Fig. 2 indicates, the left sorted cells were more tumorigenic than the right sorted cells in C3H/He mice. These results were highly significant by χ² test. Tumors developing in mice inoculated with right sorted cells also grew more slowly. The data shown in Fig. 2 were pooled from several experiments carried out at different in vitro passage levels after sorting. There was no dependence of tumorigenicity on the number of passages after sorting; this is consistent with the finding that the MHC phenotype is relatively stable (16).

**Figure 1.** IFN-γ-induced H-2 Kk and I-Ak expression on 369L and -R cells. IFN-γ-treated cells were stained with mouse mAbs to H-2 Kk (TIB 95/11.4.1) or H-2 Ak (TIB92) under saturating conditions as described in detail elsewhere (12). FITC goat anti–mouse Ig (Cappel Laboratories, Malvern, PA) was used as the second layer. Stained cells were analyzed on a FACSStar flow cytometer (Becton Dickinson & Co., Mountain View, CA). (Solid line) I-Ak, 369L; (spaced dots) I-Ak, 369R; (close dots) H-2 Kk, 369L; (dashed line) H-2Kk, 369R.

**Figure 2.** Tumorigenicity of 369L and -R in syngeneic euthymic mice. Graded numbers of cells were inoculated into C3H/He mice, and the percentage of mice developing tumors was recorded. Hatched bars indicate 369L and open bars indicate 369R. Numbers in parentheses indicate the total number of mice inoculated.

**Figure 3.** Tumorigenicity of 369L and -R in athymic nu/nu mice. Threshold numbers of cells were inoculated into nude mice, and the percentage of mice developing tumors was recorded as a function of time. Open circles indicate 369L and closed circles indicate 369R. There was a total number of 20 mice per group.
Table 1. Effect of R4-6A2 Antibody on the Growth of 369L and -R Cells in Mice

| Cells per mouse | R4-6A2 treated | Control (ND) | R4-6A2 treated | Control (ND) |
|----------------|----------------|--------------|----------------|--------------|
| 300,000        | ND             | ND           | 85 (65)        | 33 (45)      |
| 100,000        | ND             | ND           | 100 (10)       | 50 (10)      |
| 30,000         | 100 (5)        | 60 (5)       | ND             | ND           |
| 10,000         | 60 (10)        | 10 (10)      | ND             | ND           |
| 3,000          | 10 (10)        | 0 (10)       | ND             | ND           |

The data are pooled from several experiments. In some, control animals were left untreated, in others, control animals were treated with rat Ig (R4-6A2 is a rat antibody); this made no difference to tumor growth.

Tumorigenicity in Thymus-deficient nu/nu Mice. Of particular interest is the tumorigenicity of the cells in T cell-deficient mice, since one interpretation of our data is that T cells interacting with the induced class II antigens may be limiting growth in vivo of the cells. Both cell lines were markedly more tumorigenic in nu/nu mice, but at threshold inocula, it was clear that there was no significant difference (by χ² test) between the growth of class II-inducible cells and their noninducible partners (Fig. 3).

Effects on Tumorigenicity of Sorted Cell Lines of Treatment of Euthymic Mice with Antibody to IFN-γ. Treatment of C3H/He mice with the mAb R4-6A2 resulted in an increased tumor incidence in mice inoculated either with 369R or 369L cells (Table 1). The tumorigenicity of equal numbers of 369L and -R cells was directly compared in antibody-treated mice (Fig. 4). It was found that under these conditions, there was no significant difference between the two lines (by χ² test). That is to say the lower tumorigenicity of class II-inducible 369R cells normally seen in euthymic mice was abolished in mice treated with anti-IFN-γ immunoglobulin.

Lack of Correlation with Tumorigenicity of Other Properties of the Sorted Cell Lines. We have extensively studied these lines in an attempt to determine whether any other property of the cells that may influence tumorigenicity in euthymic mice correlates with the inducibility of class II antigen. We have previously reported that the amounts of ras gene product (mRNA and p21 protein product) and the IFN sensitivities are the same (16). Here, we additionally report that growth rate in vitro, anchorage dependence, sensitivity to TNF (with or without IFN-γ), and NK cytotoxicity are essentially the same in the left and right sorted lines (data not shown).

The finding that the left and right sorted lines are equally tumorigenic in nude mice, and in fact markedly more tumorigenic than in euthymic mice, implies a role for T cells in resistance to the growth of these cells in vivo; and the conclusion that it is CD4 phenotype T cells interacting via IFN-γ-induced class II antigens in the right sorted cells that determines their decreased tumorigenicity is attractive.

IFN-γ may have multiple effects on tumor cell growth in vivo; either directly, or by interaction with other components of the immune response. The observation that both 369R and 369L cells produced tumors more readily in IFN-γ-depleted mice may reflect these multiple effects of IFN. However, the finding (Fig. 4) that IFN-γ abolished the difference in tumorigenicity between these lines implies that the induction of class II antigens on the cells themselves plays a role in control of tumor growth. IFN-γ-induced class I antigens are unlikely to be important in this situation since 369R and 369L show similar levels of class I antigens after IFN treatment.

An additional pair of v-Ki-ras-transformed cell lines independently derived from C3H10T1/2 by infection with the Ki-MSV/MLV (16) gave results equivalent to those reported here for 369L and -R.

Our results stress the importance of examining not only constitutive expression of MHC antigens on cell surfaces when considering mechanisms controlling tumor growth, but also the possible modulation or induction of such antigens that may occur in vivo. Here, our observation that ras expression can abrogate class II induction (12) is particularly relevant since this, with the present results, implies a mechanism by which such ras-induced tumors may escape T cell-mediated tumor surveillance.
This work was supported by the Cancer Research Campaign.

Address correspondence to W. J. Bateman, CRC Interferon and Cellular Immunity Research Group, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK.

Received for publication 30 July 1990 and in revised form 24 September 1990.

References

1. Goodenow, R.W., J.M. Vogel, and R.L. Linsky. 1985. Histo-
compatibility antigens on murine tumours. Science (Wash. DC) 230:777.

2. Hammerling, G., D. Klar, W. Pulm, F. Momberg, and G. Mollen-
denauer. 1987. The influence of major histocompatibility complex I antigens on tumor growth and metastasis. Biochim. Biophys. Acta. 907:245.

3. Schrier, P., R. Bernards, R. Vaessen, A. Houweling, and A. van der Eb. 1983. Expression of class II major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. Nature (Lond.) 305:771.

4. Bernards, R., P. Schrier, A. Houweling, J. Bos, A van der Eb, M. Zijlstra, and C. Melief. 1983. Tumorigenicity of cells transformed by adenovirus type 12 by evasion of T-cell immunity. Nature (Lond.) 305:776.

5. Wallich, R., N. Balbuc, G. Hammerling, S. Katsav, S. Segal, and M. Feldman. 1985. Abrogation of metastatic properties of tumour cells by de novo expression of H-2K antigens following H-2 gene transfection. Nature (Lond.) 315:301.

6. Ostrand-Rosenberg, S., A. Thakur, and V. Clements. 1990. Rejection of mouse sarcoma cells following transfection of MHC class II genes. J. Immunol. 144:4068.

7. Ohnishi, K., and B. Bonavida. 1987. Regulation of lα+ retic-
ulum cell sarcoma (RCS) growth in SJL/J mice. I. Inhibition of tumour growth by passive administration of L3T4 Mab be-
fore or after tumour inoculation. J. Immunol. 138:4524.

8. Bateman, W.J., E. Jenkinson, and J. Owen. 1987. T-cell immu-
nity to murine Moloney sarcoma virus-induced tumours: L3T4+ T cells are necessary for resistance to primary sarcoma growth, but Lyt-2+ cells are required for resistance to secon-
dary tumour cell challenge. Immunology. 61:317.

9. Infanta, A.J., S. Boulware, and M. Cayle. 1988. L3T4+ T cells regulate Abelson virus-induced lymphomagenesis. J. Immunol. 140:2462.

10. Vignaux, F., and I. Gresser. 1977. Differential effects of inter-
feron on the expression of H-2K, H-2D and Ia antigens on mouse lymphocytes. J. Immunol. 118:721.

11. Wong, G., P. Bartlett, I. Clark-Lewis, F. Battye, and J. Schrader. 1984. Inducible expression of H-2 and Ia antigens on brain cells. Nature (Lond.). 310:688.

12. Maudsley, D.J., and A. Morris. 1988. Kirsten murine sarcoma virus abolishes γ interferon-induced class II but not class I major histocompatibility antigen expression in a murine fibroblast line. J. Exp. Med. 167:706.

13. Tomkins, P.T., G. Ward, and A. Morris. 1988. Role of inter-
feron gamma in T-cell responses to Semliki Forest virus infected murine brain cells. Immunology. 63:355.

14. Yeoman, H., and A. Robins. 1988. The effect of interferon gamma treatment of rat tumour cells on their susceptibility to natural killer cell, macrophage and T-cell killing. Immunology. 63:291.

15. Becker, S. 1985. IFN-gamma accelerates immune proliferation via its effect on monocyte HLA-DR expression. Cell. Immunol. 91:301.

16. Morris, A.G., G. Ward, and W. Bateman. 1989. Interaction of v-Ki-ras oncogene and interferon gamma in the control of histocompatibility antigen expression in mouse fibroblasts. Cell. Immunol. 120:470.

17. Bos, J.L., E. Fearon, S. Hamilton, M. Verlaande Vries, J. van Boom, A. van der Eb, and B. Vogelstein. 1987. Prevalence of ras gene mutations in human colorectal cancers. Nature (Lond.) 327:293.

18. Liu, E., B. Hjelle, R. Morgan, F. Hecht, and J. Bishop. 1987. Mutation of the Kirsten ras proto-oncogene in human pre-
leukaemia. Nature (Lond.) 330:186.

19. Rodenhuis, S., R. Slebos, A. Boot, S. Evers, W. Mooi, S. Wagenaar, P.C. van Bodegom, and J. Bos. 1988. Incidence and possible clinical significance of K-ras oncogene activation in adenocarcinoma of the human lung. Cancer Res. 48:5738.

20. Mann, R., R. Mulligan, and D. Baltimore. 1983. Construc-
tion of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. Cell. 33:153.

21. Norton, J., J. Connor, and R. Avery. 1984. Genesis of Kirsten murine sarcoma virus: sequence analysis reveals recombination points and leukaemogenic determinant on parental leukaemia virus. Nucleic Acids Res. 12:6839.

22. Spitalny, G.L., and E. Havell. 1984. Monoclonal antibody to murine γ interferon inhibits lymphokine-induced antiviral and macrophage tumoricidal activity. J. Exp. Med. 159:1560.