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*

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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.

Cells experimentally transformed or infected with viruses, and also some naturally occurring tumor cells, may express reduced levels of antigens of the MHC compared with "normal" cells (1–3). Since MHC antigens are essential for T cell activation, reduced expression by tumor cells could lead to their escaping immunological attack, even where there are potentially immunogenic neoantigens present. There have been several studies that have shown that increased class I expression may reduce tumorigenicity (4, 5), but little evidence of a role for class II antigens in control of tumorigenicity. However, in one experimental system, transfection of class II genes into murine tumor cells resulted in reduced tumorigenicity (6), and there have been several reports that the (class II–restricted) CD4 subset of T cells is important in the rejection of tumors in unprimed animals (7–9). The IFNs increase the expression of class I MHC antigens on many cell types (10); IFN-γ alone will additionally induce class II MHC antigen (11, 12). IFN induction of MHC class I expression in virus-infected cells or tumor cells can increase their susceptibility to killing by virus-specific CD8 (cytotoxic) T cells in vitro (13, 14). Similarly, IFN-γ induction of class II antigens may increase CD4 T cell–mediated responses (15).

We have previously reported (12) that when a line of C3H mouse fibroblasts (C3H 10T1/2) is transformed by the v-Ki-*ras* oncogene, elevation of class I antigens after IFN-γ treatment is unaffected, but the induction by IFN-γ of class II antigens is largely abolished. There remained in these transformed lines a small subpopulation of cells still inducible for class II antigen. This enabled the production of sublines (by fluorescence-activated cell sorting [FACS is a registered trademark of Becton Dickinson & Co.]) retaining the class II MHC induction shown by the parental C3H 10T1/2 line (16). We report here that these class II–inducible cells, when compared with the class II–noninducible cells, are less tumorigenic in syngeneic euthymic hosts but are of similar tumorigenicity in thymusless *nu/nu* mice or mice treated with mAb to deplete IFN-γ. Both cell lines show similar in vitro properties. We conclude that in this system one mechanism controlling tumor growth may involve expression of class II antigens by the tumor cells. A high proportion of naturally occurring human tumors exhibit *ras* gene activation (17–19); if the abrogation of induction of MHC class II antigen gene expression by *ras* occurs in human tumors, this may play a role in the development of tumors and their escape from immune recognition.

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)[1] prepared by transfection of the ψ-2 helper cell

[1] *Abbreviation used in this paper:* MSV, murine sarcoma virus.

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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% CO_2 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 mAb. 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).

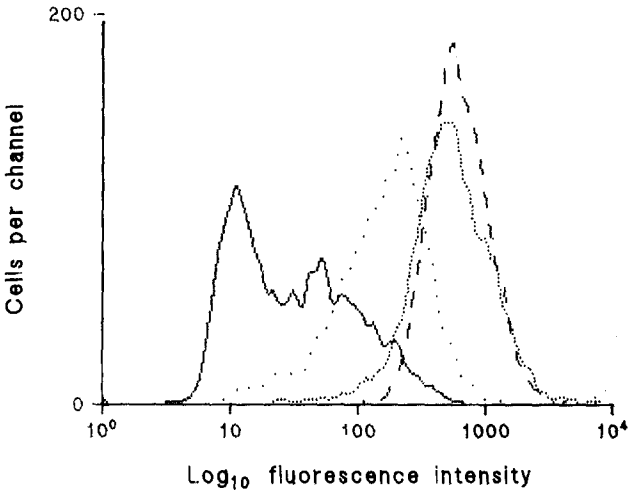

Figure 1. IFN-γ-induced H-2 Kk and I-Ak expression on 369L and -R cells. IFN-γ-treated cells were stained with mouse mAbs to H-2 Kk (TIB 95/11.4.1) or H-2 Ak (TIB92) under saturating conditions as described in detail elsewhere (12). FITC goat anti-mouse Ig (Cappel Laboratories, Malvern, PA) was used as the second layer. Stained cells were analyzed on a FACStar 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.

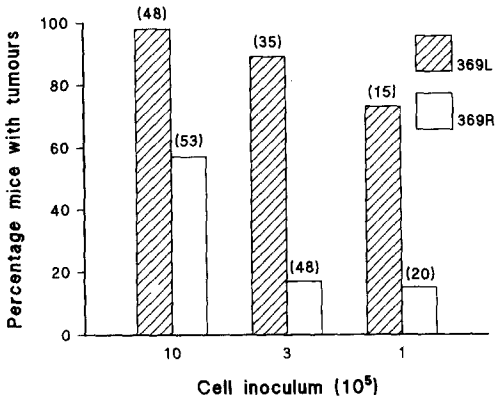

Figure 2. Tumorigenicity of 369L and -R in syngeneic euthymic mice. Graded numbers of cells were inoculated into C3H/He mice, and the percentage of mice developing tumors was recorded. Hatched bars indicate 369L and open bars indicate 369R. Numbers in parentheses indicate the total number of mice inoculated.

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 χ^2 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).

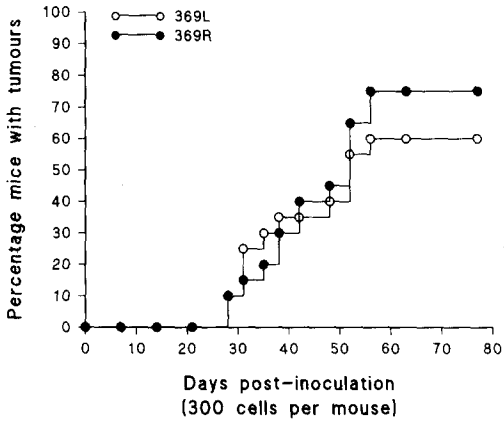

Figure 3. Tumorigenicity of 369L and -R in athymic *nu/nu* mice. Threshold numbers of cells were inoculated into nude mice, and the percentage of mice developing tumors was recorded as a function of time. Open circles indicate 369L and closed circles indicate 369R. There was a total number of 20 mice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

Table 1. *Effect of R4-6A2 Antibody on the Growth of 369L and -R Cells in Mice*

	Percent tumors (no. of mice) in mice inoculated with:			
	369L		369R	
Cells per mouse	R4-6A2 treated	Control	R4-6A2 treated	Control
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.

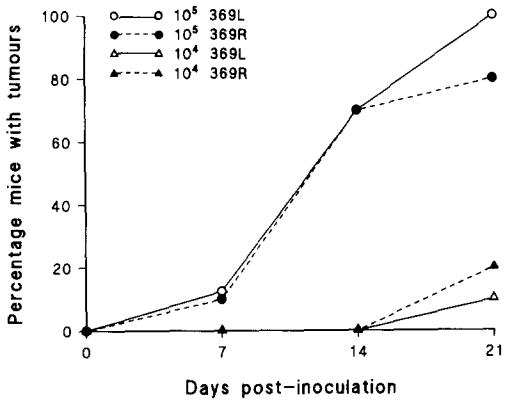

Figure 4. Tumorigenicity of 369L and -R in syngeneic mice treated with antibody to IFN-γ. Graded numbers of 369L and -R cells were inoculated into C3H/He mice treated with R4-6A2 antibody, and the percentage of mice developing tumors was recorded as a function of time. Open symbols indicate 369L and closed symbols indicate 369R, with high and low inducibility of class II antigens, respectively. (*Circles*) 100,000 cells; (*triangles*) 10,000 cells; 10 mice per group.

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 χ^2 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 χ^2 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.

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