Brief Definitive Report

KIRSTEN MURINE SARCOMA VIRUS ABOLISHES INTERFERON γ-INDUCED CLASS II BUT NOT CLASS I MAJOR HISTOCOMPATIBILITY ANTIGEN EXPRESSION IN A MURINE FIBROBLAST LINE

By D. JOHN MAUDSLEY and ALAN G. MORRIS

From the Department of Biological Sciences, University of Warwick, Coventry, CV4 7AL, United Kingdom

T lymphocytes recognize foreign antigen in the context of self MHC antigens, cytotoxic recognition usually involving class I MHC antigens and helper cell function usually involving class II. Most cells constitutively express class I antigens, although to a widely varying degree, and the levels of expression can be increased by exposure to IFNs (1). In contrast, most cell types do not express class II antigens, although in some cell types these can be induced by exposure to IFN-γ but not IFN-α or -β (2). This upregulation of MHC antigen expression by IFNs has been shown to augment T cell function, at least in vitro (e.g., reference 3), and is potentially an important component of immunity.

On the other hand, a number of viruses including adenovirus (4, 5) and Moloney murine sarcoma virus (MSV) (6), have been shown to reduce host class I MHC antigen expression levels and this may convey some benefit to the virus. The mechanism(s) involved are generally not clear. Investigation of adenoviruses has revealed that the ability of viral gene product E1A to reduce class I MHC expression correlates with oncogenicity of the virus (4, 7). It has been inferred from this that the ability of an oncogene or its product to reduce MHC antigen expression facilitates oncogenic transformation, presumably by enabling the infected cell to escape T cell–mediated tumor surveillance (5, 7).

Study of the genomic structure of the Kirsten strain of MSV reveals that most of the leukemia virus genome has been replaced by rat-derived sequences including the v-Ki-*ras* oncogene, whose human homologue is commonly associated with human tumors. We have studied the effects on MHC antigen expression of transforming/infecting fibroblasts with these viruses, and find the major effect is abolition of inducibility of class II antigens by Kirsten MSV (Ki-MSV) but apparently not by Ki–murine leukemia virus (MLV). This effect may be due to the v-Ki-*ras* oncogene.

Materials and Methods

Cells. C3H10T½ (8) and BALB/c3T3 (9) fibroblasts were obtained from the American Type Culture Collection (ATCC, Rockville, MD). C3H embryo fibroblasts were obtained by trypsin digestion of minced 15-d-old embryos. Clones of C3H10T½ cells

This work was supported by the Cancer Research Campaign of Great Britain.

were derived by limiting dilution in 96-well microtiter plates, trypsinizing wells containing single well-defined colonies. Clones were designated B9, E9, E10, F11, and G11. Virus-infected derivatives of these lines were produced by infecting the cells with helper-free MSV, MLV, or MLV/MSV after treatment with DEAE-dextran (25 μg/ml for 30 min at 37°C). ψ2 cells (10) were obtained from A. Gebhardt, Cancer Research Fund, London, U.K. All cells were cultured in Glasgow MEM with 5%-inactivated newborn calf serum.

The infected cell types used were: KC3H, KBALB, and KMEF, which were C3H10T½, BALB/c3T3, and C3H embryo fibroblasts, respectively, infected with Ki-MSV/MLV; KLVC3H and KLVBALB, which were C3H10T½ and BALB/c3T3 infected with Ki-MLV alone; C3H201 and BALB208, which were C3H10T½ and BALB/c3T3 infected with helper-free Ki-MSV.

Viruses. Stocks of Ki-MLV and Ki-MSV/MLV were derived from NIH3T3 fibroblasts producing these viruses (11). Helper-free Ki-MSV was produced by transfecting the packaging cell line ψ2 with a clone of Ki-MSV, KCC7 (12). Transfected ψ2 cells (which clearly were morphologically transformed) were grown up and supernatant was harvested. This contained about 1,000 focus-forming units of MSV per milliliter, and this was used undiluted to infect C3H10T½ and BALB/c3T3 cells to give C3H201 and BALB208 respectively. The absence of helper virus was confirmed by repeatedly harvesting supernatants from C3H201; no transforming virus was detected nor were viral antigens detectable by Western blotting using Ki-MLV–specific antisera.

IFN-γ and Induction of MHC Antigens. Recombinant murine IFN-γ (rMuIFN-γ) was produced in CHO cells using constructs as described elsewhere (13) and purified to a sp act of 10^6 U/mg. IFN titer and IFN sensitivities of different cell lines were assessed using Semliki Forest Virus (14).

Fibroblasts were treated with 10 U/ml rMuIFN-γ and 3 d later were harvested for staining for MHC antigens.

mAbs and Antisera. mAbs used against murine MHC antigens were as follows: Anti-H-2K^k: TIB95/11.4.1 (ascites diluted to 1/100); anti-H-2D^k: HB24 (purified antibody at 30 μg/ml); anti H-2K^d: 31-3-4S (Litton Bionetics Inc., Charleston, SC); anti-H-2D^d: HB19 (hybridoma supernatant); anti-H-2A^k: TIB92 (hybridoma supernatant); anti-H-2A^d: HB3 (hybridoma supernatant); and anti-H-2E^{k,d}: HB32 (hybridoma supernatant). For indirect immunofluorescence the second layer used was FITC–Fab$_2$ goat anti–mouse Ig (Cappel Laboratories, Malvern, PA) at a dilution of 1:60 in medium (Hepes-buffered RPMI, 5% FCS, 0.2% sodium azide).

Flow Cytometry. Cells were harvested and washed once in medium (Hepes-buffered RPMI containing 5% FCS and 0.2% sodium azide). Approximately 2.5×10^5 cells were incubated at 4°C for 30 min in 50 μl of antibody at appropriate dilution, or medium control, with 0.2% sodium azide. Cells were then washed and incubated at 4°C for 45 min in 50 μl FITC–Fab$_2$ goat anti–mouse Ig at 1:60. Cells were analyzed on a FACStar flow cytometer (Becton Dickinson & Co., Mountain View, CA) and fluorescence histograms were obtained from these data using the Consort 30 computer program.

Results and Discussion

Expression of MHC Antigens by Fibroblasts. MHC antigens could not be detected on the surface of C3H10T½ fibroblasts unless they were treated with IFN-γ. 10 U/ml of IFN-γ, however, induced the expression of H-2K, A, E and D, i.e. both class I and class II MHC (Fig. 1). BALB/c3T3 fibroblasts on the other hand, express low but clearly detectable levels of class I antigen without IFN treatment. Class I antigen expression was markedly increased by treatment with IFN-γ, but class II antigens were not induced.

The C3H mouse embryo fibroblasts again differed. No MHC antigens were detected on untreated cells; treatment with IFN-γ induced class I but not class II antigens.

Infection by MSV, but not MLV, Abolishes Induction of Class II, but not Class I,

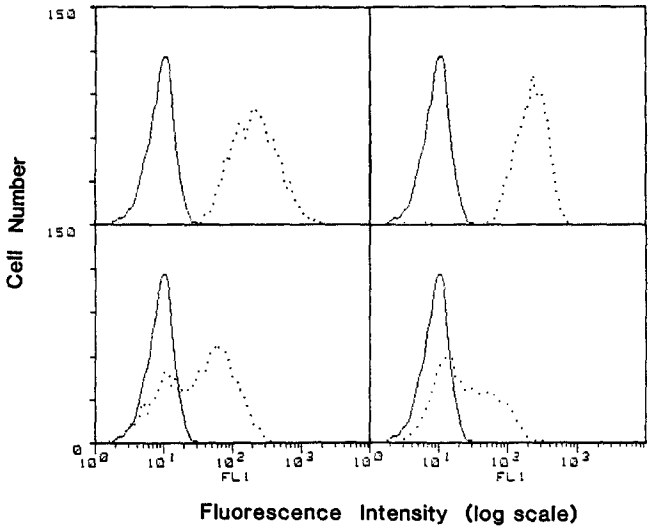

FIGURE 1. Induction of H-2^k antigens on C3H10T½ fibroblasts by IFN-γ; 3 d after treatment with 10 U/ml IFN-γ. (*Solid line*) Staining with anti H-$2A^d$ control mAb. (*Dotted line*) (*Top left*) H-$2K^k$; (*top right*) H-$2D^k$; (*bottom left*) H-$2A^k$; and (*bottom right*) H-$2E^k$.

MHC Antigens. Of the various fibroblasts described above, only BALB/c3T3 showed MHC antigen expression in the absence of IFN-γ, although all three were inducible for class I antigens, and infection with Ki-MLV, -MSV, or both did not cause a significant reduction in this constitutive expression. The effects of infection of all three cell types with Ki-MLV/MSV complex on IFN-γ–induced class I expression appeared to be minimal, although the consistent reductions observed suggest it may be real; and likewise infection of C3H10T½ or BALB/c3T3 with either MLV or MSV (alone) had minimal effects on class I expression.

C3H10T½ was alone, in that class II MHC antigens were induced by IFN-γ; however, they could not be induced on derivatives of C3H10T½ infected with Ki-MLV/MSV complex (Fig. 2, *top panels*). Whether this was due to the MLV or to the MSV, or to a combination of the two, can be assessed by the effects of each virus independently on C3H10T½. Class II antigens were clearly induced on KLVC3H (MLV-infected C3H10T½) in a way similar to the parental C3H10T½ (Fig. 2), strongly suggesting that the inhibition of class II MHC expression was the function of Ki-MSV.

To test further whether Ki-MSV was responsible and whether it could inhibit class II expression on its own, C3H10T½ cells were infected with helper-free Ki-MSV. These C3H201 cells, like KC3H, could not be induced for class II MHC antigen expression (Fig. 2), showing MSV to be responsible for this inhibition. To ensure that the failure of class II induction was not due simply to changes in IFN sensitivity, the MSV-infected cells were subjected to antiviral assay; the results indicated that there had been no change in sensitivity (data not shown). However, to exclude completely this possibility, a dose-response experiment was carried out with C3H201. In this it was found that H-2K expression was nearly maximal at 10 U/ml IFN-γ and no increase occurred beyond 100 U/ml; however, even at 1,000 U/ml no induction of H-2A occurred.

It is clear then that it is Ki-MSV and not Ki-MLV that is inhibiting the

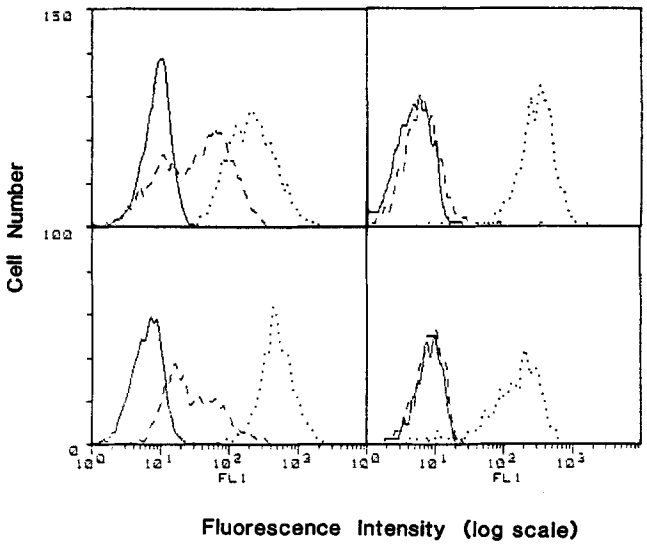

FIGURE 2. Induction of class I and class II MHC antigens on C3H10T½ and virally infected derivatives. (*Top left*) C3H10T½. (*Top right*) KC3H (Ki-MLV/MSV infected); (*Bottom left*) KLVC3H (Ki-MLV infected); (*Bottom right*) C3H201 (Ki-MSV alone). (*Solid line*) H-2A^d; (*dotted line*) H-2K^k; (*dashed line*) H-2A^k.

induction of MHC antigens. Since both MLV and MSV share the same long terminal repeat (LTR) regions (12), it is unlikely that these are involved, and more probably it is the v-Ki-*ras* gene (the only gene encoded by Ki-MSV) that is responsible.

This has clear implications on tumor immunity, where it may be that part of the tumorigenic potential of v-Ki-*ras* resides in its ability to inhibit IFN-induced increases in MHC antigen expression, at least in some cell types. As expected, C3H201 readily produced tumors in vivo in syngeneic C3H/HeJ mice, while the parent line C3H10T½ could not. Similar mechanisms could apply to other oncogenes like adenovirus E1A, which in oncogenic adenoviruses has been shown both to reduce MHC expression and to be oncogenic (7). It is not clear at this stage whether we are observing a direct effect of *ras* or an effect of a downstream phenotypic change consequent upon *ras* expression.

Five Clones of C3H10T½ All Express Class II Antigens. It can be observed above that the levels of class II MHC antigen expression on IFN-γ–treated C3H10T½ covered a broad range. Although the C3H10T½ cell line has been cloned previously this raises the albeit unlikely possibility that it contains subpopulations of cells that respond to IFN-γ with differing levels of class II antigen expression. If this is the case, Ki-MSV could be transforming preferentially the unresponsive cells, thus giving the results above.

To test this, C3H10T½ was cloned by limiting dilution and single colony wells were chosen for analysis. Five clones were treated with 10 U/ml IFN-γ and stained for H-2 antigens 3 d later. All clones showed inducibility for class I (H-2K and D) and class II (H-2A and E) MHC antigens. Some small variation between clones was seen in levels of expression. Most of these clones (4/5) exhibited a broad spread of H-2A and H-2E expression very similar to the parent line. The fifth clone, F11, exhibited a noticably higher level of expression of H-2A and H-2E than the others, with slightly less of a spread in levels. F11 also expressed H-2K at a higher level than the other clones. Expression of H-2K and

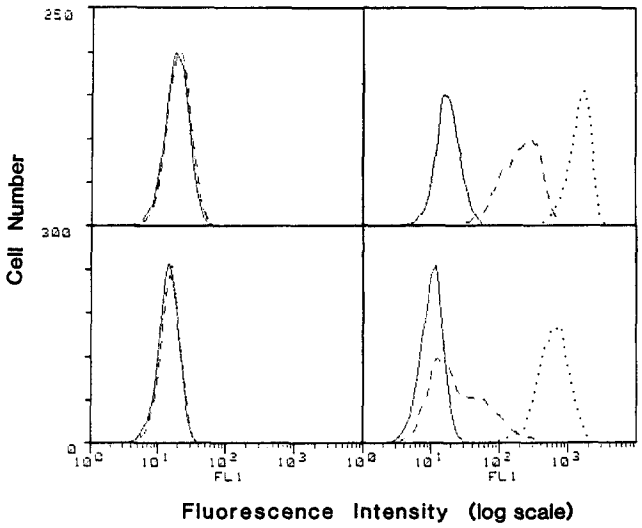

FIGURE 3. Induction of H-2K and H-2A on C3H10T½ clones F11 (*top*) and B9 (*bottom*). (*Left panels*) Without IFN, (*right panels*) after 3 d with 10 U/ml IFN-γ. (*Solid line*) FITC conjugate alone; (*dotted line*) H-2K^k; (*dashed line*) H-2A^k.

H-2A on IFN-γ–treated and untreated F11 and B9 (the clone showing lowest levels of H-2A expression) is shown in Fig. 3.

First, the spread seen in class II antigen expression by the clones confirms that the similar spread seen for C3H10T½ was due to an intrinsic property of the cell line and not (at least to any significant degree) to nonresponsive subline(s) within the population. Second, it is noteworthy that all clones analyzed expressed clear levels of class II MHC antigen after exposure to IFN-γ, suggesting again that C3H10T½ is at least reasonably homogeneous in its response to IFN-γ. In addition, it is interesting that one clone was isolated that expressed a higher level of class II antigen.

These results are good evidence against the slight possibility of MSV selectively transforming uninducible cells and therefore support the conclusion that Ki-MSV inhibits the induction of class II antigens in C3H10T½ cells that it transforms.

Summary

The effect of infecting fibroblasts with Kirsten murine sarcoma virus/murine leukemia virus (Ki-MSV/MLV) on constitutive and IFN-γ–induced H-2 antigen expression was investigated. The fibroblasts used were two established cell lines (C3H10T½ and BALB/c3T3) and fresh embryo fibroblasts from C3H mice. Class I antigens were expressed constitutively by BALB/c3T3; infection with MLV, MSV or the two together had little effect on this constitutive expression. Class I antigens (H-2K, H-2D) were strongly induced on all three types of fibroblast by rIFN-γ, and infection had little effect on this. None of the fibroblasts expressed constitutively detectable levels of class II antigen; however, C3H10T½ fibroblasts could be induced for both H-2A and H-2E by IFN-γ. Infection of C3H10T½ with helper-free Ki-MSV, or MSV together with MLV, completely abolished this induction of class II antigens, while infection with MLV alone had

little effect, implying that the abolition of class II induction was due to genomic regions of Ki-MSV not shared with Ki-MLV, probably the v-Ki-*ras* gene.

Received for publication 27 October 1987 and in revised form 2 December 1987.

References

1. Vignaux, F., and I. Gresser. 1977. Differential effects of interferon on the expression of H-2K, H-2D and Ia antigens on mouse lymphocytes. *J. Immunol.* 118:721.
2. Kelly, V. E., W. Fiers, and T. B. Strom. 1984. Cloned human interferon-γ, but not interferon-β or -α, induces expression of HLA-DR determinants by foetal monocytes and myeloid leukaemic cell lines. *J. Immunol.* 132:240.
3. Morris, A., P. T. Tomkins, D. J. Maudsley, and M. Blackman. 1987. Infection of cultured murine brain cells by Semliki Forest Virus: effects of interferon-$\alpha\beta$ on viral replication, viral antigen display, major histocompatibility complex antigen display and lysis by cytotoxic T lymphocytes. *J. Gen. Virol.* 68:99.
4. Schrier, P. I., R. Bernards, R. T. M. J. Vaessen, A. Houweling, and A. J. van der Eb. 1983. Expression of class I major histocompatibility antigens switched off by highly oncogenic adenovirus 12 in transformed rat cells. *Nature* (*Lond.*). 305:771.
5. Andersson, M., S. Pääbo, T. Nilsson, and P. A. Peterson. 1985. Impaired intracellular transport of class I MHC antigens as a possible means for adenoviruses to evade immune surveillance. *Cell.* 43:215.
6. Flyer, D., S. Burakoff, and D. Faller. 1985. Retrovirus-induced changes in major histocompatibility complex antigen expression influences susceptibility to lysis by cytotoxic T lymphocytes. *J. Immunol.* 135:2287.
7. Bernards, R., P. I. Schrier, A. Houweling, J. L. Bos, A. J. van der Eb, M. Zijlstra, and C. J. M. Melief. 1983. Tumourigenicity of cells transformed by adenovirus type 12 by evasion of T cell immunity. *Nature* (*Lond.*). 305:776.
8. Rezinkoff, C. A., D. W. Brankow, and C. Heidelberger. 1973. Establishment and characterisation of a cloned line of C3H mouse embryo cells sensitive to post confluence inhibition of division. *Cancer Res.* 33:3231.
9. Aaronson, S. A., and G. J. Todaro. 1968. Development of 3T3-like lines from BALB/c mouse embryo cultures: transformation susceptibility to SV40. *J. Cell. Physiol.* 72:141.
10. Mann, R., R. C. Mulligan, and D. B. Baltimore. 1983. Construction of a retrovirus packaging mutant and its use to produce helper-free defective retrovirus. *Cell.* 33:153.
11. Morris, A., C. Clegg, J. Jones, B. Rodgers, and R. J. Avery. 1980. The isolation and characterisation of a clonally related series of murine retrovirus-infected mouse cells. *J. Gen. Virol.* 49:105.
12. Norton, J. D., J. Connor, and R. J. Avery. 1984. Genesis of Kirsten murine sarcoma virus: sequence analysis reveals recombination points and potential leukaemogenic determinant on parental leukaemia virus genome. *Nucleic Acids Res.* 12:6839.
13. Morris, A., and G. Ward. 1987. Production of recombinant interferons by expression in heterologous mammalian cells. *In* Interferons and Lymphokines: A Practical Approach. M. Clemens, A. Morris, and A. Gearing, editors. IRL Press: Oxford. 61–71.
14. Atkins, G. J., M. D. Johnston, L. M. Westmacott, and D. C. Burke. 1974. Induction of interferon in chick cells by temperature-sensitive mutants of Sindbis virus. *J. Gen. Virol.* 25:381.