Interferon-mediated enhancement of metastasis. Are MHC antigens involved?

PIER-LUIGI LOLLINI, CARLA DE GIOVANNI, BRUNELLA DEL RE, GIORDANO NICOLETTI, GIORGIO PRODI and PATRIZIA NANNI

Istituto di Cancerologia, Università di Bologna, Viale Filopanti 22, I-40126 Bologna, Italy

(Received 19 June 1986; accepted 8 December 1986)

The relationship between major histocompatibility complex (MHC) antigens and metastasis was investigated on B16 melanoma variants. B16 cell lines express low amounts of murine MHC (H-2) antigens. A high expression can be induced in line B16-A by in vitro treatment with immune interferon (IFN-gamma) or by in vivo transplant in allogeneic mice. The increase of H-2 antigens correlated with an enhancement of lung colonization in young syngeneic mice. The higher metastatic capacity of B16-A cells with induced high levels of H-2 antigens was observed also in adult mice and in young mice pretreated with cyclophosphamide. These results were confirmed investigating the behaviour of a mutant B16 clone (B78H1) which was selectively resistant to the H-2-inducing action of IFN-gamma: lung colonization ability was not increased by IFN pretreatment. The study of variants derived from individual B16-A lung colonies revealed a wide range of H-2 levels. Variants with a low expression had a low colonization ability; one out of two variants with a high H-2 expression also was poorly colonizing. IFN-gamma-mediated H-2 expression appeared to act as an enhancer, rather than a determinant of B16 metastatic capacity.

Introduction

The role played by major histocompatibility complex (MHC) antigens in the pathogenesis of cancer metastasis has not yet been precisely clarified. Several types of metastatic human tumors show alterations (usually a decrease) of MHC class I antigens expression [1, 2, 3, 8, 13, 16], but no general correlation has yet been proposed with metastatic spread or prognosis [22]. The data obtained so far with murine tumors [4, 6, 24] concordantly show that the treatment of metastatic cells with interferons induces a concomitant increase of H-2 expression and of metastatic potential.

Two different explanations of this phenomenon have been proposed. The data obtained by Feldman and co-workers with Lewis lung carcinoma [4] and T10 fibrosarcoma [11] indicate that different class I regions could have antagonistic effects on the control of metastasis by T lymphocytes. In contrast, Taniguchi et al. [24], studying B16 melanoma, found an inverse relationship between global H-2 expression and metastasis on the one side and NK sensitivity of tumour cells on the other.

We have recently characterized the expression of H-2 antigens in various B16 cell lines [14] and we have shown that the reduction of H-2 expression observed in vitro can be reversed by interferon-gamma (IFN-gamma) treatment or by in vivo passage in allogeneic mice [12]. We present here data on the influence of H-2 antigens on the
metastatic ability of several B16 variants differing from each other in H-2 expression or interferon sensitivity. Their metastatic potential has been compared using both normal and NK-depressed mice.

Materials and methods

Mice
C57BL/6NCrlBR (C57BL/6) and BALB/cAnNCrlBR (BALB/c) male mice were purchased from Charles River, Calco, Italy. Mice used for lung colonization assay were either 8–12 weeks old (hereafter referred to as young mice) or 20–25 weeks old (referred to as adult mice) or young mice treated with a single intraperitoneal administration of cyclophosphamide (240 mg/kg, Asta Werke, Bielefeld, F.R.G.) four days before the injection of B16 cells (referred to as young CY-treated mice).

Cells
B16-A cell line has been previously characterized [12, 14]. Clone B78H1 [7] was obtained through the courtesy of Dr T. Boon, Ludwig Institute, Brussels, Belgium.

Interferons
Natural mouse IFN-gamma was a kind gift of S. Landolfo, Istituto di Microbiologia, Turin, Italy. Recombinant IFN-gamma was obtained through the courtesy of G. R. Adolf, Ernst-Börhringer Institut, Wien, Austria. The antiproliferative activity of IFN was measured adding increasing concentrations of recombinant IFN-gamma to B16 cells seeded 24 h before into Falcon (Oxnard, U.S.A.) T25 flasks; each day one control and one IFN-treated flask were harvested and the cell yield determined.

In vitro and in vivo induction of H-2 antigens
In vitro, cells were treated with 50 U/ml of IFN-gamma for 24 h (except when otherwise stated), then processed for quantitative absorption or for lung colonization assay. The protocol used to induce H-2 expression in B16 cells by means of in vivo transplant has been described [12]; briefly, allogeneic BALB/c mice were inoculated subcutaneously with $10^6$ cells; one month later the ensuing tumour was dissociated mechanically and enzymatically, and the resulting cells were cultured in vitro for 2–4 passages to get rid of normal cells.

Absorption assay
Quantitative in vitro absorption of complement-dependent cytotoxicity was carried out as described [12, 14]. The following anti-H-2 reagents were used: H-142-23, monoclonal anti-H-2K, working dilution 1 : 55–1 : 60, purchased from Biotest, Milan, Italy, and E-2, monospecific anti-H-2D, working dilution 1 : 10, obtained from the NIH, Bethesda, MD, U.S.A. Cytotoxic activity against C57BL/6 lymph-node cells was assessed in a trypan blue dye exclusion assay.

Lung colonies
C57BL/6 mice were inoculated with $2.5 \times 10^4$–$10^5$ cells in the tail vein (i.v.). Mice were killed 21 days later and their lungs were removed and fixed. The lungs of mice