Species specificity of a novel factor which augments the expression of MHC class I antigens on tumor cell lines

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Abbreviations: MHC, major histocompatibility complex; MHC-AF, MHC augmenting factor; IFN- γ , interferon γ ; Con-A, concanavalin-A; PBS, phosphate-buffered saline

Abstract

A novel factor which augments the expression of major histocompatibility complex I (MHC augmenting factor or MHC-AF) antigens on tumor cell lines, has been isolated from the culture supernatants of human peripheral blood mononuclear cells activated by concanavalin-A. A mouse equivalent of this factor has also been isolated from the culture supernatants of mouse spleen cells activated by mitogens or in a mixed lymphocyte reaction. Mouse MHC-AF enhances the expression of class I MHC antigens on murine tumor cell lines (EL-4 and BW5147) but not on human tumor cell lines (K562 and HR-7). Human MHC-AF on the other hand enhances the MHC I expression on both human as well as murine cell lines. Interferon gamma (IFN- γ), a cytokine also known to enhance the expression of MHC I antigens, acts in a highly species specific manner with mouse IFN- γ augmenting the MHC I on murine tumor cell lines and human IFN- γ augmenting the MHC I on human tumor cell lines only. These results indicate important differences in the cross species biological activities of MHC-AF and IFN- γ , and provide additional evidence for MHC-AF being distinct from IFN- γ .

Keywords: major histocompatibility complex, lymphokines, lymphocytes, interferon gamma

Introduction

Major histocompatibility complex (MHC) antigens play a central role in interactions within immune cells and between cytotoxic lymphocytes and tumor cells (Zinkernagel and

Doherty, 1979; Karre *et al.*, 1986; Saxena, 1997). Factors which modulate expression of MHC antigens can be potent immunomodulators. Many factors including interferon (Piontek *et al.*, 1985; Wiebke *et al.*, 1990), phorbol esters and sodium butyrate (Dokhelar *et al.*, 1982, 1984), are known to augment class I MHC expression on tumor cells. Work from our laboratory has described a putative novel cytokine termed MHC augmenting factor (MHC-AF), which enhances the class I MHC expression on tumor cells and renders them resistant to NK lysis (Saxena, 1987; Saxena *et al.*, 1988, 1989, 1992, 1996; Puri, 1995; Raval *et al.*, 1997). Interferon γ (IFN- γ) has activities similar to MHC-AF, and since it is also produced by mitogen activated lymphocytes, it is possible that the MHC-AF is same as IFN- γ . Biological and biochemical properties of MHC-AF, however, strongly suggest that it is distinct from IFN- γ (Saxena *et al.*, 1987, 1988, 1992). MHC-AF unlike IFN- γ , is resistant to pH 2 treatment and lacks anti-viral activity (Dokhelar *et al.*, 1984; Saxena *et al.*, 1988). While the final proof of MHC-AF being different from IFN- γ will come when the former is purified to homogeneity, this has posed problem since MHC-AF is produced in extremely low quantities which makes its purification difficult. We have devised purification schemes utilizing liquid chromatography, isoelectric focusing and ionexchange and reverse phase HPLC steps, but MHC-AF has not yet been purified to homogeneity (Saxena, 1987; Saxena *et al.*, 1988, 1992, 1996; Raval *et al.*, 1997). Recently, we have isolated a MHC-AF activity from culture supernatants of murine spleen cells activated by mitogen or in a mixed lymphocyte reaction (Saxena *et al.*, 1992; Puri, 1995). Having obtained MHC-AF preparations from two species, we have explored the species specificity of the biological activity of MHC-AF and compared it from that of IFN- γ . Our results indicate a major difference in species specificities between MHC-AF and IFN- γ .

Materials and Methods

Culture medium

Tumor cell lines were maintained in RPMI 1640 from Sigma (St. Louis, MO), supplemented with 5% fetal calf serum, 20 μ M 2-mercaptoethanol, 300 μ g/ml glutamine, and 60 μ g/ml gentamicin (complete medium). For generation of MHC-AF, the lymphocyte cultures were carried out in commercially available serum free medium, DCCM-2 from Biological Industries, Israel, supplemented with 20 μ M 2-mercaptoethanol, 300 μ g/ml glutamine and 60 μ g/ml gentamicin.

Cytokines

Murine recombinant IFN- γ was obtained as a gift from Pittsburgh Cancer Institute. Human recombinant IFN- γ was obtained from Genzyme (Cambridge, MA).

Monoclonal antibodies and antibody conjugates

Supernatants from hybridoma cell lines B468 (secreting anti-H-2K^k), B896 (secreting anti-H-2K^b) and HB95 (secreting anti-HLA-A, B and C) were used as sources of monoclonal antibodies. Hybridoma cell lines B486 and B896 were kindly provided by Dr. Eli Gorelik of the Pittsburgh Cancer Institute and hybridoma HB95 was obtained from ATCC (Rockville, MD, USA). Goat anti-mouse Ig-FITC from Gibco BRL was used as second antibody in flow cytometric studies.

Tumor cell lines

Murine tumor cell lines EL-4 (haplotype H-2^b, from ATCC, TIB39) and BW5147 (haplotype H-2^k, from ATCC, TIB47) are T cell lymphomas originally obtained from NIA, NIH (courtesy of Dr. W. H. Adler). Human tumor cell lines, K562, a chronic myelogenous leukemia (ATCC, CCL 243) was also obtained from NIA, NIH, and HR7, a gastric carcinoma cell line was originally obtained from Pittsburgh Cancer Institute (courtesy of Dr. T. L. Whiteside).

Preparation of MHC-AF

Human MHC-AF was purified from the culture supernatant of human peripheral blood lymphocytes (HPBLs) activated by concanavalin A (Con-A), as described before (Saxena *et al.*, 1996; Raval *et al.*, 1997). Mouse MHC-AF preparations were derived from culture supernatants of mouse spleen cells activated in a mixed lymphocyte reaction (Saxena *et al.*, 1981), by using the purification scheme utilized for rat MHC-AF (Saxena *et al.*, 1988). Human MHC-AF has previously been shown to lack any IFN like anti-viral activity (Saxena *et al.*, 1996). Murine MHC-AF preparations were also devoid of any anti-viral activity assessed by using a L-cells VSV system of IFN bioassay (Saxena *et al.*, 1988).

Estimation of cell surface antigen on tumor cell lines

The tumor cells in culture were treated with IFN- γ (500 U/ml) or MHC-AF preparations (50 μ g/ml) for 2 to 3 day, and stained for class I MHC antigens as described before (Saxena *et al.*, 1988, 1996; Raval *et al.*, 1997). Briefly, control or treated tumor cells were suspended in 50 μ l of hybridoma supernatants containing relevant anti-MHC class I antibodies and incubated for 20 min at 4°C. Cells were washed once with cold phosphate-buffered saline (PBS) and suspended in 50 μ l of goat anti-mouse immunoglobulin FITC at 1:400 dilution. After 20 min. incubation at 4°C, cells were washed once with cold PBS and fixed in 0.5 ml of 1% (w/v) paraformaldehyde in PBS. Stained and fixed cells were analyzed in a Coulter Epics-XL flow

Figure 1. Effect of murine and human MHC-AF and IFN- γ on MHC I expression of murine tumor cell lines. Two murine cell lines, EL-4 (Panel A) and BW5147 (Panel B) were cultured alone (a) or with mouse MHC-AF (b), or mouse IFN- γ (c), or human IFN- γ (d), or human MHC-AF (e) for 48 h. After two days, tumor cells were harvested, stained for class I MHC antigen expression as described in Materials and Methods, and analyzed on a flow cytometer.

Figure 2. Effect of murine and human MHC-AF and IFN- γ on MHC I expression of human tumor cell lines. Two human cell lines, K562 (Panel A) and HR7 (Panel B) were cultured alone (a) or with mouse MHC-AF (b), or mouse IFN- γ (c), or human IFN- γ (d), or human MHC-AF (e) for 48 h. After two days, tumor cells were harvested, stained for class I MHC antigen expression as described in Materials and Methods, and analyzed on a flow cytometer.

cytometer.

Results

Effect of recombinant IFN- γ and MHC-AF on the expression of MHC class I antigens on two murine tumor cell lines

EL4 and BW5147 murine cell lines were treated with mouse or human IFN- γ or with human or mouse MHC-AF preparations for two days, and stained for expression of MHC class I antigens. Results of flow cytometric analysis in Figure 1 show that mouse IFN- γ induced significant increase in the expression of class I MHC antigens on both mouse tumor lines, whereas human IFN- γ failed to have any effect on MHC I expression. Both human as well as mouse MHC-AF however increased MHC class I antigen expression on both tumor cell lines.

Effect of recombinant IFN- γ and MHC-AF on the expression of MHC class I antigens on two human tumor cell lines

K562 and HR7 human tumor cell lines were treated with mouse or human IFN- γ or with human or mouse MHC-AF preparations for two days, and stained for expression of MHC class I antigens. Results of flow cytometric analysis in Figure 2 show that mouse IFN- γ as well as mouse MHC-AF had no effect on the expression of class I MHC antigens on both K562 and HR7 tumor lines. Human IFN- γ as well as human MHC-AF, however, increased MHC class I antigen expression on both tumor cell lines.

These results indicate that the biological activity of

IFN- γ is strictly species specific since mouse and human IFN- γ acted on mouse and human cell lines only. In case of MHC-AF, however, mouse MHC-AF was species specific in action whereas human MHC-AF was biologically active on both mouse and human cell lines.

Discussion

We have previously reported partial purification and characterization of a rat MHC-AF (Saxena, 1987; Saxena *et al.*, 1988). The rat factor is a 12-kDa protein with an isoelectric pH of 4.8 (Saxena *et al.*, 1988). Recently, partial purification and characterization of human MHC-AF has also been reported and it appears to be a 30-kDa protein with pI of 6.0 (Saxena *et al.*, 1996; Raval *et al.*, 1997). Using the purification scheme standardized for rat MHC-AF, we have now been able to partially purify a mouse MHC-AF from culture supernatants of mouse spleen cells activated in mixed lymphocyte reaction (Puri, 1995). Human and mouse MHC-AF are assayed by their effect on the expression of MHC class I antigens on human and mouse tumor cell lines respectively. Since IFN- γ is also active in MHC-AF assays and activated T lymphocytes (e.g. in mitogen activated spleen cells and in mixed lymphocyte reaction) produce IFN- γ , that raises the question whether MHC-AF is identical to IFN- γ or somehow related to IFN- γ . Based upon biological, immunological and biochemical characterization of MHC-AF preparations, we feel convinced that MHC-AF is a new cytokine distinct from IFN- γ (Saxena, 1987; Saxena

et al., 1988, 1989, 1992, 1996; Puri, 1995; Raval *et al.*, 1997). Final confirmation of a distinct identity of MHC-AF will come when this factor is purified to homogeneity and its structure determined. Since MHC-AF is produced in extremely small quantities, its final purification is yet to be achieved. In our present study, we have used partially purified MHC-AF preparations from mouse and human sources. These MHC-AF preparations were, however, devoid of any IFN- γ contamination as assessed by anti-viral bioassay of IFN. We tested for the augmentation of MHC class I expression on two mouse and two human tumor cell lines in response to IFN- γ and MHC-AF from the two species. While both mouse and human IFN- γ and mouse MHC-AF were strictly species specific in their biological activities, human MHC-AF acted in a species non-specific way, since it increased the MHC class I expression on both mouse and human cell lines. Differences in the species specificity patterns of MHC-AF and IFN- γ provide an important fresh evidence which indicate that the two mediators are distinct from each other.

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