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Partial purification and characterization of a novel human factor that augments the expression of class I MHC antigens on tumour cells

R K SAXENA*†, Q B SAXENA, T L WHITESIDE, R H GOLDFARB, and R B HERBERMAN

Pittsburgh Cancer Institute, University of Pittsburgh School of Medicine, Pittsburgh, PA 15213, USA

*School of Life Sciences, Jawaharlal Nehru University, New Delhi 110067, India

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Abstract. A cytokine which augments the expression of major histocompatibility complex (MHC) I antigens on K562 and gastric carcinoma tumour (HR) cells, has been isolated from the culture supernatant of Concanavalin-A (Con-A) activated human peripheral blood mononuclear cells. The factor, termed MHC augmenting factor (MHC-AF) has been partially purified by Sephadex G-100 column chromatography, preparative isoelectric focusing and HPLC with ion-exchange as well as sizing columns. MHC-AF activity is associated with a 35 kDa molecule which has pI of 6·0. Interferon (IFN)-α, ß, tumour necrosis factor (TNF), Interleukin (IL)-2, IL-4, IL-5 and IL-7 had no significant effect in MHC-AF bioassay, but IFN-γ had significant MHC-AF activity. Antibodies to IFN-α, IFN-ß and TNF-α did not block the activity of MHC-AF, but anti-IFN-y antibodies could partially neutralize the activity. However, unlike IFN-γ, MHC-AF activity was resistant to pH 2·0 treatment. Purified MHC-AF preparations did not have any activity in WISH cell/encephalo myocarditis virus (EMC) IFN bioassays. In addition, anti-IFN-y affinity column did not retain MHC-AF activity. These results indicate that a MHC-AF distinct from IFN-γ, is produced by activated human mononuclear cells.

Keywords. Cytokine; MHC-AF; class I MHC; interferon; NK-RIF.

1. Introduction

Class I and class II molecules encoded in the major histocompatibility complex (MHC) play a central role in immune cell interactions. The helper subset of T-lymphocytes recognize antigens presented by the class II MHC antigens on antigen presenting cells (Rosenthal and Shevach 1973). Class I MHC molecules associate with and present antigenic peptides to the cytotoxic T-lymphocytes (CTL) (Zinkernagal and Doherty 1979). Antigen receptors on CTL recognize antigenic peptides on target cells, in association with class I MHC antigens, as a result, CTL-mediated target lysis is restricted in activity by class I MHC molecules as well as antigens. Natural killer (NK) cells comprise another class of cytotoxic lymphocytes which can lyse allogeneic and even xenogeneic target cells (Sarin and Saxena 1989), and the class I or II MHC antigens are not required for NK recognition of target cells. Nonetheless, alternations in the quantitative expression of class I MHC antigens on some target cells have been shown to change their susceptibility to NK cell mediated lysis. An inverse relationship has often been observed between the amount of the class I MHC antigen expressed on

†Corresponding author (Fax, 091-11-6865886; Email, R K Saxena @jnuniv.ernet.in).

tumour cells and their susceptibility to NK or lymphokine activated Killer (LAK) cells (Harel-Bellan *et al* 1986; Storkus *et al* 1987), though, exceptions to this trend have also been reported (Gorelik *et al* 1988; Leiden *et al* 1989).

Both NK as well as T cells participate in immunesurveillance mechanisms and, since the class I MHC antigen expression on tumour target cells may determine their susceptibility to these immunesurveillance mechanisms, factors which regulate the expression of the class I MHC antigens on tumour cells, may determine the fate of a tumour *in vivo.*

Many factors are known to modulate class I MHC antigen expression on tumour cells. These include cytokines such as IFN and TNF (Wiebke *et a1*1990), *in vivo* passage of tumour cells (Piontek *et al* 1985) and treatment with inducers of differentiation, such as phorbol esters and sodium butyrate (Dokhelar *et a1*1982, 1984). We have described the presence of a factor in culture supernatants of rat spleen cells, which enhances class I MHC antigen expression on YAC tumour cells and renders them resistant to NK cells (Saxena 1987; Saxena *et al* 1989). This factor which was termed NK lysis resistance including factor (NK-RIF) has been partially purified and characterized (Saxena 1987; Saxena *et al* 1988a,b, 1989, 1992). Rat NK-RIF is distinct from IFN, since the former has no anti-viral activity and its activity is not neutralized by anti-IFN antibodies (Saxena *et al* 1988a,b). In the present communication, we report the partial purification and characterization of a human factor which augments the expression of class I MHC antigens on some tumour cell lines. The factor termed MHC-AF is a 35 kDa molecule, with a pI of 6·0 and appears to be distinct from IFN or other known cytokines.

2. Materials and methods

2.1 *Reagents*

Various cytokines used in this study were obtained as gifts from the following sources: IL-2 was kindly provided by Cetus Chiron (Emeryville, CA); IL-12, Hoffmann LaRoche (Nutley, NJ, USA); IL-4 and IL-7 by sterling Drug Inc. (Malvern, PA, USA); IL-6 by Sandoz (Vienna, Austria); IFN-γ by Roussel CLAF (Romainville, France); and TNF-α by Knoll Pharmaceuticals (Whippany, NJ, USA). The hybridoma cell lines HB99 (secreting anti class I MHC Mab) and HB8291(secreting anti human IFN-γ Mab) were obtained from American Type Cell Culture (ATCC), Bethesda, Md, USA. Reagents for affinity column were purchased from BioProbe, International Ltd., Tustin, Ca, USA. Reagents for gel filtration and isoelectric focusing were obtained from Sigma (St. Louis, Mo, USA).

2.2 *Culture supernatants from Con-A—activated human peripheral blood mononuclear cells (HPBMC)*

HPBMC were isolated by Ficoll Hypaque density gradient centrifugation (Boyum 1974). Cells derived from Leukaphereses of normal blood donors (Central Blood Bank, Pittsburgh, PA, USA) were diluted 20-fold in cold RPMI 1640 medium and 20 ml alliquots were layered on Lymphoprep solution (density 1·077 g/ml, Sigma) and centrifuged at 600*g* for 15 min. HPBMCs were removed from the interphase, washed three times with RPMI medium and cultured at a concentration of 5 x 10^6 cells/ml, in RPMI-1640 medium supplemented with 5% fetal calf serum (FCS), glutamine (300 μg ml) and 50 μg ml penicillin-streptomycin (complete medium, CM). Con-A

(Sigma, St Louis, Mo, USA) was added at a final concentration of 5 μg/ml and supernatants were collected by centrifugation (2000 *g* for 15 min) after 3 days.

2.3 *Initial processing of Con-A culture supernatants*

Con-A supernatants were generally processed in batches of 1 to 4 liters. For each liter of supernatant maintained at 4°C, 599 g of ammonium sulphate was added gradually and the supernatant allowed to stand overnight at 4°C. Precipitated proteins were pelleted by centrifugation at 10,000 *g* for 20 min and resuspended in 0·1 M glycine-HCl buffer pH 2·0. The solution was dialyzed against the pH 2·0 buffer for two days and then neutralized to pH 7·0 by using I0 N NaOH. Any insoluble material present at this stage was removed by centrifugation, and the solution was concentrated on an Amicon 3K membrane filter to about 30 ml for each 1 liter of the starting material.

2.4 *Sephadex column chromatography*

A column of Sephadex G-100 (5 x 115 cm) was used and in each run 30 ml of the concentrates obtained by the processing of the culture supernatant, was fractionated on the column. The column was run at 4°C at a flow rate of 1ml/min using phosphate buffered saline (PBS).

2.5 *Preparative isoelectric focusing* (*IEF*)

Fractions of Sephadex G-100 column, with MHC-AF activity were pooled, concentrated and dialyzed against 1% glycine and fractionated by IEF by the procedure described elsewhere (Saxena 1987). IEF fractions were dialyzed against PBS and assayed for MHC-AF activity. Active fractions were pooled and concentrated using Amicon 3K membrane filters.

2.6 *HPLC fractionation*

An analytical anion-exchange HR8 HPLC column (Waters) and a sizing HPLC column PP125 (Waters) were used along with a Waters system HPLC equipment. Prior to fractionation on a HR8 column, the pooled IEF fractions with MHC-AF activity were dialyzed against 10 mM Tris-HCl buffer pH 8·2. The material absorbed on the column was eluted in a gradient of 20 to 100% (v/v) of 0·5 N NaCI in 10 mM Tris-HCl buffer pH 8·2. Column fractions were filter sterilized and assayed for MHC-AF activity. Aliquots (0·2 ml) of the material eluted from the column and containing MHC-AF activity were then applied to a PP125 column and PBS was used as eluting buffer. Fractions were assayed for MHC-AF activity after filter sterilization.

2.7 *MHC-AF bioassay*

HR cell line derived from a gastric carcinoma metastatic to the liver and established in our laboratory (Shimizu *et al* 1991) was specially sensitive to the effect of MHC-AF. In the present study, we have used both K562 and HR cells interchangeably for the bioassay of MHC-AF activity. The tumour cells cultured at 0·1 x 10^6 cells/ml in RPMI-1640 CM

(for K562) or MEM CM (for HR cells), in the presence of the desired concentrations of the test agents, for 2 to 3 days. At the end of the culture, cells were harvested and stained for class I MHC antigens. Culture supernatant of HB99 hybridoma was used as a source of a mouse monoclonal antibody (Mab) reactive to a non-polymorphic region of class I MHC antigens. Control or treated cell pellets were suspended in 50μl of the HB99 culture supernatants and incubated for 15 min at 4°C. Cells were washed once with cold PBS and suspended in 50μl of rabbit anti mouse Ig-FITC at 1: 100 dilution. After 15 min incubation at 4°C, cells were washed and fixed on 0·1 ml of a 1% (w/v) para-formaldehyde in PBS. Stained and fixed cells were analysed in flowcytometer. HR or K562 cells treated with second antibody alone did not show significant nonspecific staining. For K562 tumour cells, the data are presented as the percentage of cells becoming positive for class I MHC antigens after MHC-AF treatment. For HR cells, which show low but significant basal expression of class I MHC antigens, the increase in the expression of class I MHC antigens was assessed by the increase in the mean fluorescence intensity (MFI).

2.8 *IFN bioassay*

Anti-viral bioassay for IFN was performed using WISH cell/EMC system by the procedure described before (Saxena *et al* 1988b). Briefly, 3 x 10^4, WISH cells in 0·2 ml of MEM complete medium, were plated in 96-well fiat-bottom tissue culture plates, in the presence of desired concentrations of the test agents. After 20 h, 10 μl of an appropriately diluted EMC virus preparation was added to each assay well. After two days, when untreated WISH cells were totally lysed by EMC, the level of protection in treated WISH cells was assessed under the microscope. One unit of IFN corresponded to half protection of WISH cells. For better quantitation, WISH cells were stained with 1% crystal violet in 70% ethanol as described before (Saxena *et al* 1988b). After washing the wells, dye was eluted in 0·2 ml of methanol and the absorbance read in an ELISA reader using a 540 nm filter.

2.9 *Anti-IFN affinity column*

Anti-human IFN-γ monoclonal antibodies were isolated from the culture supernatant of hybridoma HB 8291 (ATCC), by using Avid Al affinity column (BioProbe, International Ltd., Tustin, Ca, USA). This antibody was coupled to a solid base in a cartridge: column using Hydrazide Avid Chrom cartridge system (Bio Probe International Ltd., Tustin, Ca, USA). A total of 2·5 mg of antibody was immobilized on the column, which could theoretically bind 10 x 10^6 units of IFN-γ. Efficacy of this column to remove IFN-γ was tested by placing different amounts of IFN-γ on the column. The highest amount of IFN-γ tested (10 units) was totally retained by the column. The column could be used repeatedly after removing the bound material by washing with glycine-HCl buffer at pH 2·0 and reequilibrating the column in PBS.

3. Results

3.1 *Partial purification of MHC-AF*

We previously described a rat factor which induced NK resistance in YAC tumour cells and enhanced the expression of class I MHC antigens on these cells (Saxena 1987;

Saxena *et al* 1988a, 1989, 1992). This factor termed NK-RIF was purified from the culture supernatants of ion-A activated rat spleen cells. In order to look for a possible human counterpart of the rat factor, we fractionated the Con-A supernatant of HPBMC. Con-A supernatants were processed and concentrated as described in § 2 and then fractionated on a Sephadex G-100 column. All fractions were examined for their effect on MHC I antigen expression on HR tumour cells. Figure 1 indicate the elution profile of the human MHC-AF from a typical experiment. MHC-AF activity eluted towards the end of the major albumin peak. Last few fractions with MHC-AF activity had 1 to 5 mg protein per fraction. Fractions with MHC-AF activity were pooled, concentrated, dialyzed against 1% glycine for further fractionation by preparative IEF.

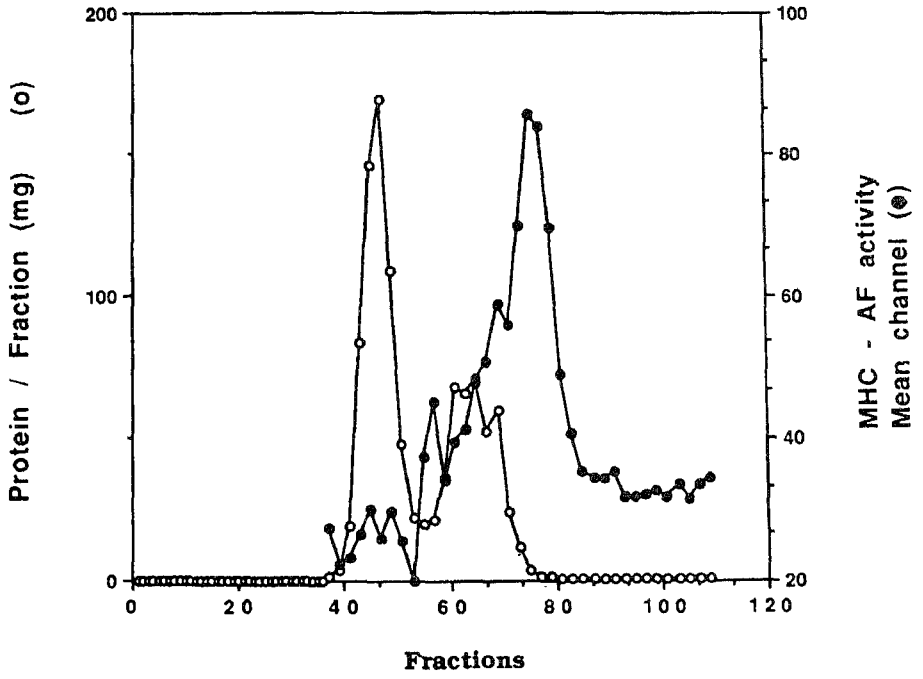

Figure 1. Fractionation of human MHC-AF on a Sephadex G100 olumn. Culture super natant (0·9 liters) of Con-A—activated HPBMC, was processed as described in § 2. The concentrate (30 ml) obtained after pH 2·0 dialysis step and containing 1·1g of protein was fractionated on a Sephadex G100 column (5 x 115 cm) using PBS. Fractions of 320 drops were collected and assayed for protein by measuring absorbance at 280 nm (O). Each fraction was also tested (at 10% v/v) for MHC-AF activity using HR tumour cells as described in §2(●).

Results in figure 2 show a typical IEF profile of MHC-AF. It should be noted that dilute fractions obtained from preparative IEF were assessed as such, thus the magnitude of effect of MHC-AF is not very high. The isoelectric pH of MHC-AF appears to be 6·0. The IEF fractions with MHC-AF activity were pooled, dialyzed against 0·1 M Tris-HCl buffer, pH 8·2, concentrated and placed on a HR8 ion-exchange HPLC column. This column was developed with a gradient of 20 to 100% v/v of 0·5 M NaCl. Figure 3 shows that the MHC-AF activity eluted from the HR8 column along with the very first protein peak. In order to assess the molecular weight of MHC-AF, fractionation on a PP125 sizing HPLC column was used. The elution profile of the MHC-AF activity on PP125 column is shown in figure 4. The PP125 column was

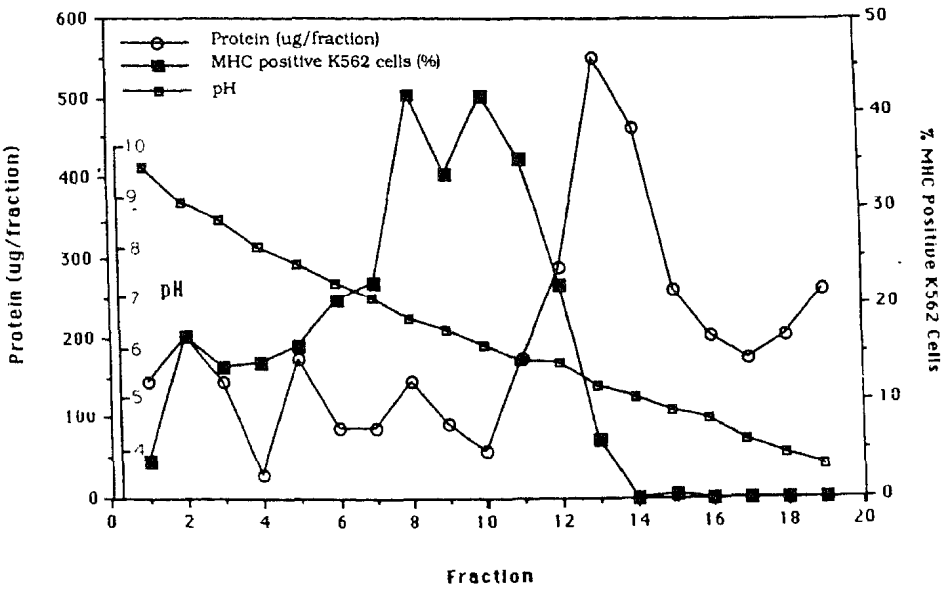

Figure 2. Preparative isoelectric focusing of MHC-AF activity. Fractions with MHC-AF activity obtained after the Sephadex G100 column were pooled, concentrated and dialyzed against a 1% glycine solution and fractionated on a flat bed preparative isoelectric focusing set up using granulated gel. Each fraction was dialyzed against PBS and tested for MHC-AF activity at 5% v/v using K562 tumour cells.

calibrated with different protein markers and from the position of the MHC-AF activity peak, its molecular weight appears to be around 35 kDa. SDS page analysis of the pooled activity peak from PP125 column showed several protein bands indicating that the MHC-AF preparation was not homogeneous at this stage.

3.2 *Relationship of MHC-AF to known cytokines*

Many cytokines, such as IFN and TNF are known to enhance the class I MHC antigen expression on a variety of tumour cell lines. Dose response and time kinetics of MHC-AF activity were compared-with those of IFN and TNF. Figures 5 and 6 show that TNF and IFN-α and ß had little if any MHC I augmenting effect on HR cells. IFN-γ and MHC-AF has similar dose response and time kinetics pattern, although at the concentrations tested, MHC-AF appeared to be more potent than IFN-γ. Some other cytokines like IL-2, 4, 6 and 7 were also examined and found to be devoid of MHC-AF activity.

Con-A is known to induce IFN-γ production by lymphocytes. However, the protocol for purification of MHC-AF included a step of extensive dialysis against pH 2·0, a treatment known to destroy the IFN-γ activity. Moreover, using the WISH cell/EMC system, no IFN-like anti-viral activity could be detected in MHC-AF preparations. We considered the possibility that pH 2·0 treatment could have abolished the anti-viral but not the class I MHC augmenting effect of IFN-γ. It was crucial to assess this point, since MHC-AF activity might in that case, represent a pH 2·0 "inactivated" IFN-γ. To test this possibility, human recombinant IFN-γ was dialysed for two days against 0·1 M glycine-HCI buffer, pH 2·0 and then examined for both

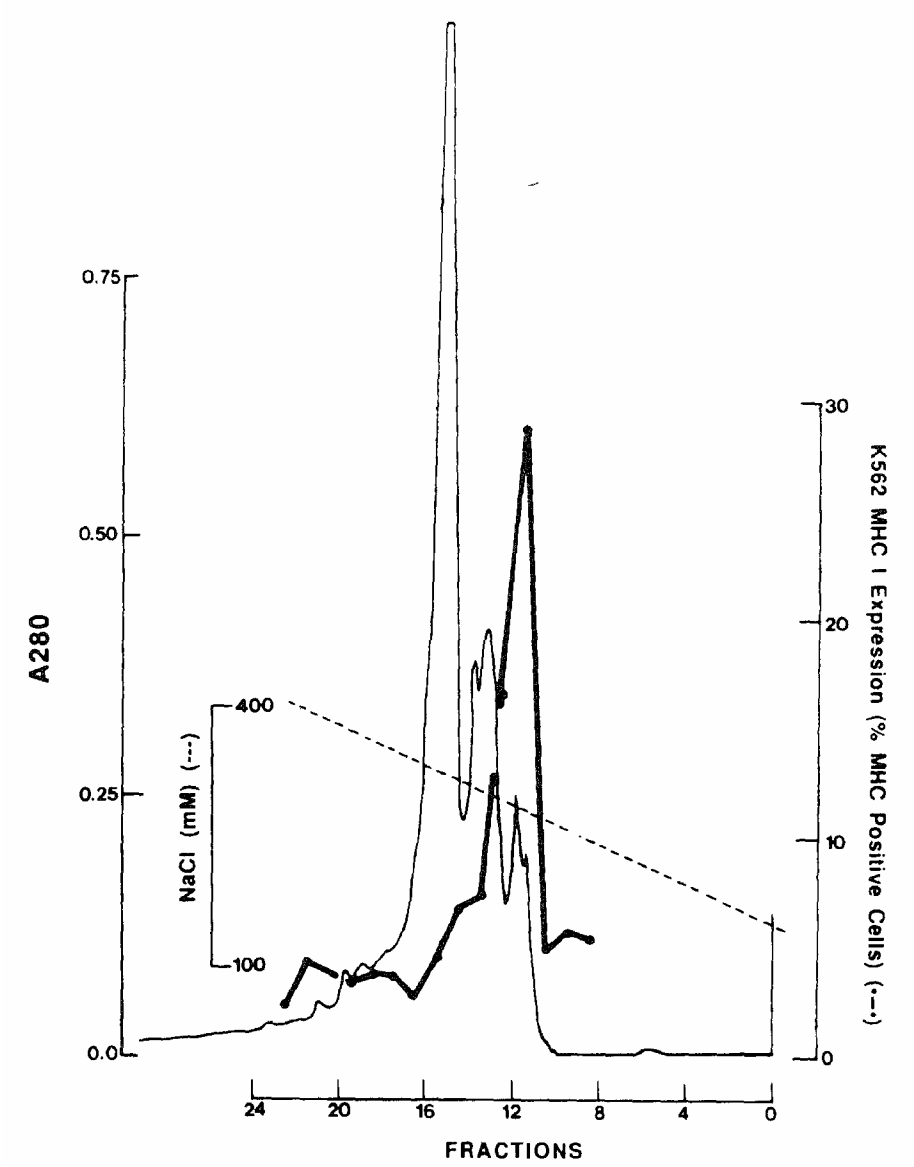

Figure 3. Fractiontion of MHC-AF activity on HPLC anion exchange column. An IEF-purified MHC-AF preparation was fractionated on an anion exchange HPLC column run in 10 mM Tris HCl buffer pH 8·2, using a sodium chloride gradient from 20 to 100% of 0·5 M NaCI (brocken line). Elution profile of proteins is indicated by thin line. Fractions were tested for MHC-AF activity on the HR cell line at 10% v/v as described in §2 and the profile of the activity is shown in bold line.

anti-viral and class I MHC augmenting activities. These experiments indicated that pH 2·0 treatment resulted in a parallel loss (about 95% loss in each assay) of antiviral and class I MHC augmenting activity of IFN-γ (results not shown). These results rule out the possibility that MHC-AF activity represented a pH 2·0 altered form of IFN-γ.

To further evaluate the possible relationship of MHC-AF to known cytokines, attempts were made to neutralize the MHC-AF activity with antibody preparations capable of

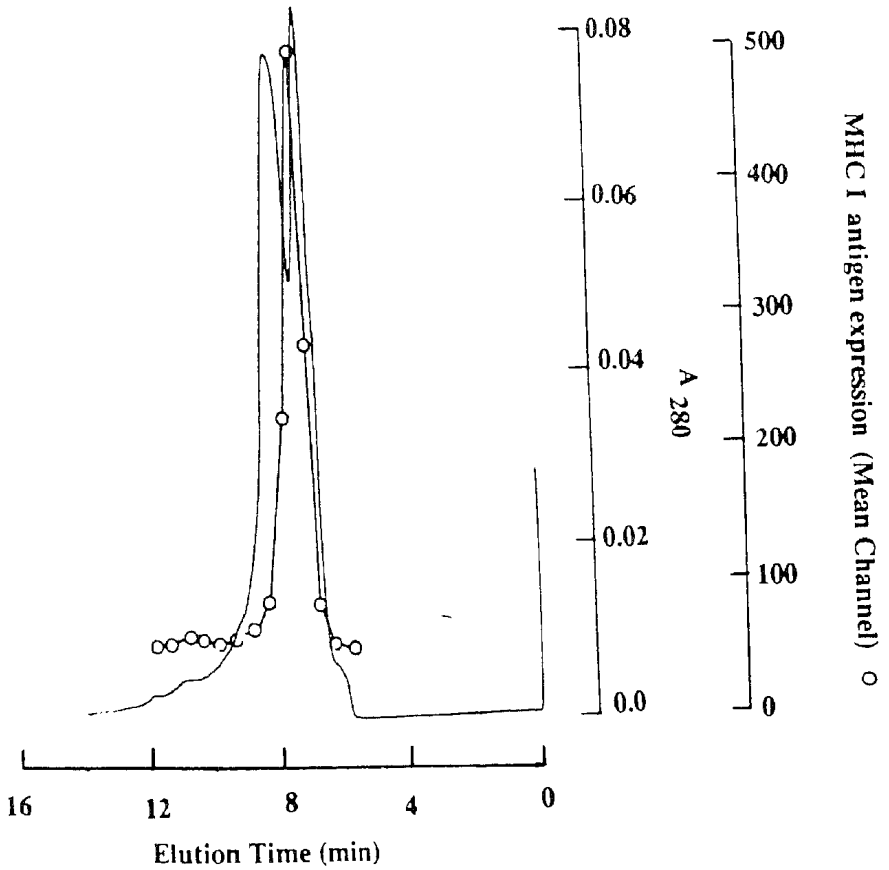

Figure 4. Fractionation of MHC-AF activity on HPLCPP125 column. 0·2 ml of a MHC-AF preparation (400 μg/ml) obtained after the IEF step was fractionated on a PP125 HPLC column and the fractions were examined for MHC-AF activity (O) at 10% v/v, using HR cells as described in § 2.

neutralizing various types of interferons or TNF. Results of a typical experiment in table 1 show that the antibody preparations effectively neutralized the biological activities of their respective cytokines. Antibodies to IFN-α and ß and to TNF had no effect on the biological activity of MHC-AF. Anti IFN-γ had a partial blocking effect on MHC-AF activity (table 1). Using both anti-IFN-γ and anti-TNF antibodies did not further reduce the effect of MHC-AF preparation. These results indicate that of the cytokines examined, MHC-AF activity might have some relationship with IFN-γ. Alternatively, the MHC-AF preparation tested might have contained some IFN-γ activity as well as MHC-AF activity. This issue was further examined by using an anti-IFN-γ affinity column. This affinity column was made by immobilizing 2·5 mg of an anti IFN-γ monoclonal antibody and a demonstrated capacity to absorb at least 10^5 units of IFN-γ. Results in figure 7 indicate that the potency of MHC-AF preparation in enhancing class I MHC expression on HR tumour cells was not reduced by passage through anti-IFN-γ affinity column. Affinity column passed MHC-AF also has no IFN-γ contamination as assessed by biological and immunological assays. MHC-AF activity, therefore, may not be ascribed to the presence of IFN-γ.

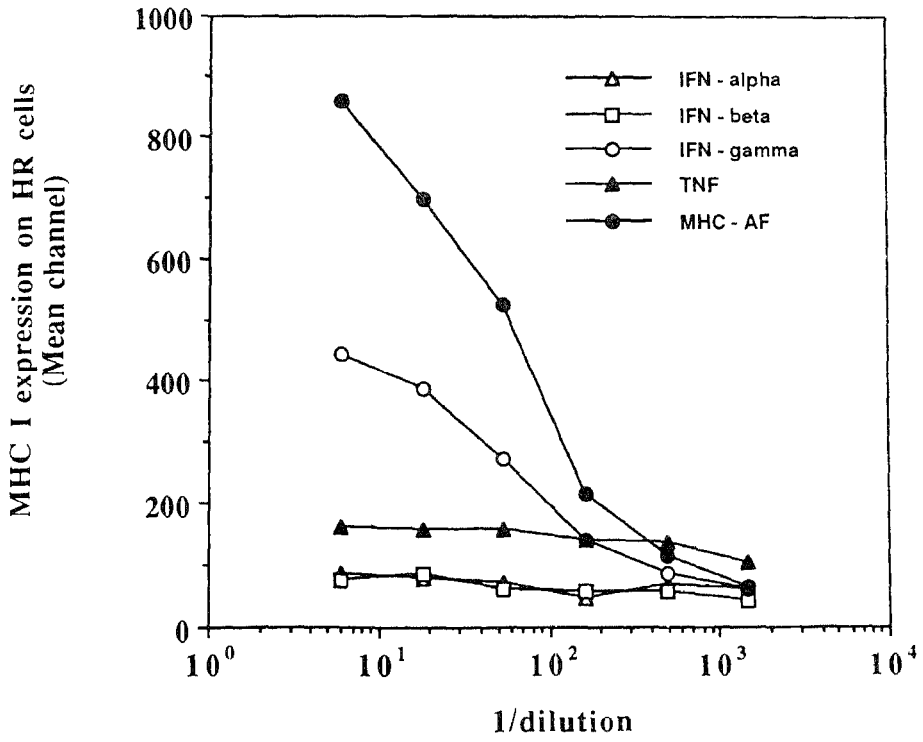

Figure 5. Dose response effect of IFNs, TNF and MHC-AF on class I MHC antigen expression on HR cells. HR tumour cells (0·1 x 10^6/ml) were cultured with various concentra tions of IFNs, TNF or MHC-AF preparations. The highest concentration points in the figure correspond to 666 U/ml of IFN-α, *ß* or γ, 0·5 *μ*g/ml of TNF-α and 10 *μg/ml* of a MHC-AF preparation obtained from a HR8 HPLC column. After 2 days of culture, HR cells were stained for class I MHC antigens and analysed on a flow cytometer.

4. Discussion

We report here the partial purification and characterization of a factor from the culture supernatants of Con-A activated HPBMSs, which augments the expression of class I MHC antigens on tumour cells. A similar class I MHC expression-augmenting factor was previously reported to be released by mitogen-activated rat spleen cells (Saxena 1987; Saxena *et al* 1988a, 1989, 1992). The rat factor was, however, initially identified by its property of inducing NK resistance in YAC tumour cells, and this bioassay was used for its purification and characterization (Saxena *et al* 1988a). For this reason, the rat factor was named as NK-RIF. Purified preparations of rat NK-RIF were also found to augment the expression of class I MHC antigen on YAC tumour cells (Saxena et *al* 1988a, 1992). While looking for the human equivalent of the rat factor, we used MHC I augmentation as a primary bioassay and, therefore, have referred to it as MHC-AF rather than NK-RIF. Purified MHC-AF preparation could induce NK resistance in K562 tumour cells (results not shown). although we cannot be certain at present whether a single molecule is responsible for MHC-AF and NK-RIF activities.

Human MHC-AF was partially purified on gel filtration followed by preparative IEF and further purification on HPLC ion-exchange and sizing columns. The molecular

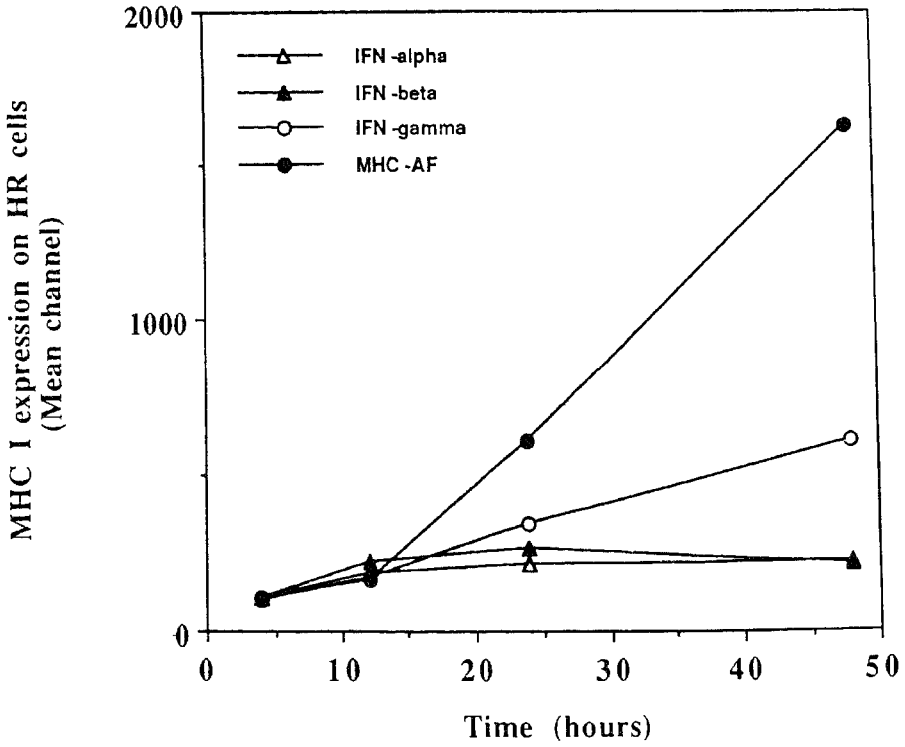

Figure 6. Kinetics of the effect of MHC-AF and IFNs on the expression of class I MHC antigens on HR tumour cells. HR tumour cells were initiated in culture at 0·1 x 10^6 cells/ml. Various IFNs (400 U/ml) or MHC-AF preparation obtained from a HR8 HPLC column (1 μg/ml) were added to the cultures at different time points so that at the time of harvesting (48 h), cells had been exposed to the agents for varying periods of time. HR cells were stained for the class I MHC antigen expression and analysed by flowcytometer.

weight of MHC-AF was around 35 kDa with a pI of 6·0. As indicated in results, MHC-AF preparation obtained after final purification step of PP125 column, was still not homogeneous. We think that the presence of fetal calf serum in the initial culture medium introduces a large amount of a complex mixture of irrelevant proteins, which makes it difficult to obtain a homogeneous preparation of MHC-AF. It will be necessary to devise procedure for generating this factor in serum free medium in order to get a homogeneously pure MHC-AF. Attempts are currently underway to standardize procedure for generating MHC-AF in serum free medium.

The crucial issue is whether MHC-AF is one of the known cytokines or a novel factor. IFN and TNF can augment the expression of class I MHC antigens on tumour cells (Weber and Rosenberg 1988; Mortarini *et al* 1990). IFN-α and ß as well as TNF-α are, however, relatively poor inducers of MHC antigens. Moreover, neutralizing antibodies to TNF and IFN-α or ß, had no effect on the class I MHC augmenting activity of MHC-AF preparations. These results would seem to rule out IFN-α and ß and TNF-α as entities responsible for the MHC-AF activity. The possible relationship of MHC-AF to IFN-γ was of particular concern. IFN-γ is known to most effectively induce MHC I antigen expression on tumour cells. Moreover, antibodies to IFN-γ could at least partially neutralize the MHi-AF activity. Even though the MHC-AF preparations were devoid of any activity in anti-viral bioassays for IFNs and

Table 1. Effect of antibodies to IFN or TNF on MHC-AF activity.

Test agent	Test antibodies	K562 MHC I expression (% positive)
—	—	1·5
MHC-AF	—	98·6
IFN-α	—	36·8
—	Anti-IFN-α	7·0
IFN-α	Anti-IFN-α	7·8
MHC-AF	Anti-IFN-α	96·2
IFN-β	—	46·2
—	Anti-IFN-β	10·3
IFN-β	Anti-IFN-β	11·6
MHC-AF	Anti-IFN-β	96·8
IFN-γ	—	88·6
—	Anti-IFN-γ	11·2
IFN-γ	Anti-IFN-γ	16·3
MHC-AF	Anti-IFN-γ	62·6
TNF-α	—	30·6
—	Anti-TNF-α	3·7
TNF-α	Anti-TNF-α	4·4
MHC-AF	Anti-TNF-α	93·0

K562 tumour cells were treated with various combinations of reagents for 3 days under conditions described in §2. All IFNs and TNF were used at 100 U/ml concentration and all antisera were added to a concentration sufficient to neutralize 500 U/ml of their respective cytokine. A MHC-AF preparation obtained by IEF was used at a final concentration of 10 μg/ml. The tumour cells were examined for expression of class I MHC antigens by flowcytometry.

the purification protocol of MHC-AF included a step of pH 2·0 dialysis for 2 days, a treatment which is known to inactivate IFN-γ, the possibility exsited that the pH 2·0 treatment abolished the antiviral activity of IFN-γ in Con-A supernatants without influencing its MHC augmenting activity. This crucial issue was, therefore, examined by evaluating the antiviral as well as the class I MHC augmenting effects of IFN-γ, before and after pH 2·0 dialysis. Our results indicated that pH 2·0 treatment destroyed both these activates of IFN-γ. The activity of MHC-AF preparations could, therefore, not be attributed to the presence of pH 2·0-exposed IFN-γ. In addition, an affinity column with immobilized Mabs to IFN-γ with proven efficacy to absorb IFN-γ, did not show any significant absorption of MHC-AF activity, even though the affinity column-assed MHC-AF preparation had no biologically or immunologically active IFN-γ. Taking these data together, it appears quite unlikely that IFN-γ is responsible for the potent MHC I expression augmenting activity in MHC-AF preparations. Nonetheless, in view of an apparent partial neutralization of MHC-AF activity by anti-IFN-γ antibodies, it is possible that the effect of MHC-AF may, at least partially, be mediated through IFN-γ or some other factor that is cross-reactive with IFN-γ. Definitive resolution of this issue will require further characterization of MHC-AF.

The class I MHC antigen expression on tumour cells *in vivo* may be an important factor in determining the susceptibility of these cells to immunosurveillance mechanisms.

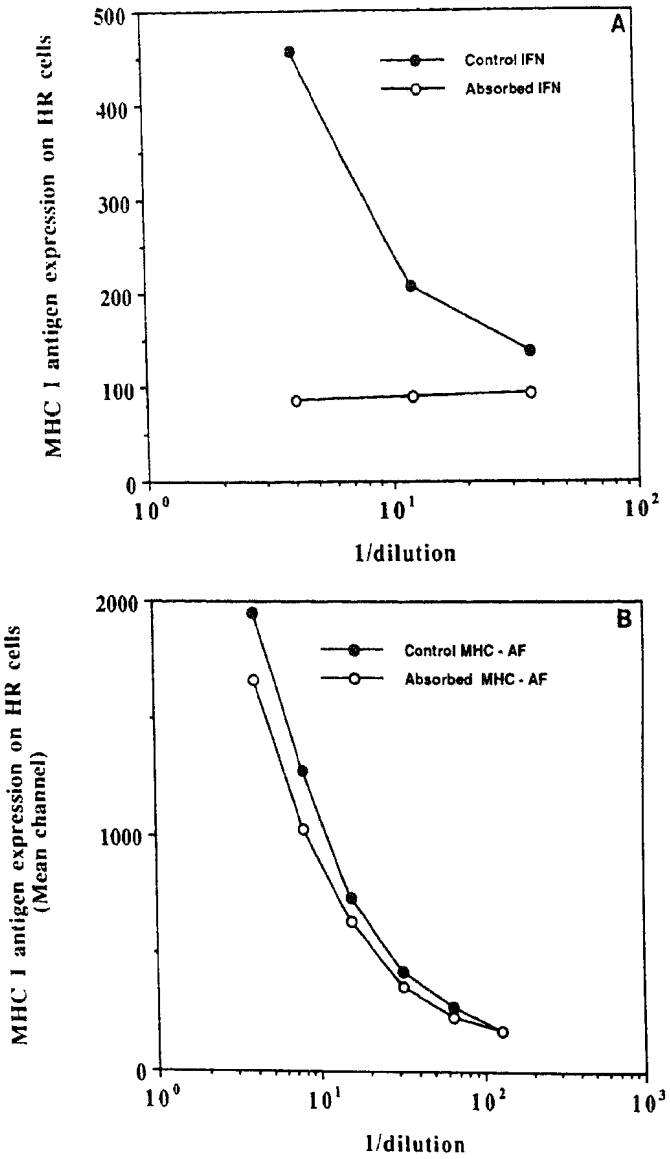

Figure 7. Retention of IFN-γ but not of human MHC-AF on a human IFN-γ affinity column. One ml of human recombinant IFN-γ preparation (10,000 U/ml) was passed through an anti-IFN-γ affinity column. A MHC-AF preparation obtained after the HR8 HPLC fractionation (24 μg/ml) was also passed through the affinity column. Preparations of IFN (**A**) or MHC-AF (**B**) before (●) and after (O) absorption on the affinity column, were tested at various concentration, for their effect on class I MHC antigen expression on HR tumour cells as described in § 2.

Moreover, the immuno-therapeutic potential of experimental infusion of *in vitro* expanded tumour infiltrating lymphocytes (TILs), might be enhanced by protocols which would sustain, or even boost the expression of class I MHC antigens on tumour cells *in vivo*. In this context, cytokines like TNF and IFN alone or in combination are currently being examined for their class I MHC boosting property (Weber and

Rosenberg 1988; Mortarini *et al* 1990). MHC-AF is a potent inducer of class I MHC antigen expression *in vitro.* It will be of interest to examine the efficacy of MHC-AF for tumour class I MHC expression augmentation *in vivo.*

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Corresponding editor: INDIRA NATH