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Enhanced MHC I antigen expression on tumour target cells is inversely correlated to lysis by allogenic but not by xenogenic NK cells

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Abstract. Relationship between the levels of MHC class 1 antigen expressed on tumour cells and their susceptibility to allogenic and xenogenic NK cells was investigated. Mouse and human natural killer-resistance inducing factor (NK-RIF) preparations were used for augmenting/inducing MHC 1 antigen expression on murine YAC and human K562 tumour cells, respectively YAC cells with augmented MHC I antigen expression became relatively resistant to lysis by murine NK cells but not to rat NK cells. Similarly, induction of MHC I antigens on K562 cells reduced their susceptibility to human NK cells but not to monkey NK cells. These results indicate that the inverse correlation of MHC I antigen expression and NK susceptibility does not hold true for xenogenic pairs of NK effector and target cells.

Keywords. NK cells; MHC antigens; tumour cells; natural killer-resistance inducing factor; xenogenic targets.

1. Introduction

Unlike cytotoxic T cells, target cell lysis mediated by NK effector cells is not restricted by MHC class I antigens (Herberman and Ortaldo 1981). MHC antigens may nonetheless play an important role in determining the NK susceptibility of tumour cells. An inverse correlation between the levels of MHC I antigens on target cells and their NK-susceptibility has been shown (Harel-Beilan *et al* 1986; Storkus *et al* 1987; Welsh *et al* 1981). Augmentation of MHC class I antigens induced by cytokines (Welsh *et al* 1981; Piontek *et al* 1985; Saxena *et al* 1988), *in vivo* passage of tumour cells (Taniguchi *et al* 1985; Karre *et al* 1986) or transfection with MHC class I genes (Storkus *et al* 1989), have been shown to depress NK-susceptibility of target. In addition, certain mutants with depressed or no expression of MHC I antigens show an increased susceptibility to NK lysis (Ljunggren and Karre 1985). This inverse relationship however does not always hold (Gorelik *et al* 1988; Nishimura *et al* 1988; Sarin *et al* 1994).

While NK effector-target interaction per se is not restricted by MHC antigens, it is not very clear if the inverse correlation of MHC I antigen expression and NK susceptibility, is influenced by qualitative disparities at MHC loci on NK effector and target cells. Allogenic differences on NK and target cells do not appear to be important in this regard since enhanced MHC expression is known to render

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targets NK resistance irrespective of the MHC allotypes of the two cell populations. In the present study we have investigated the MHC and NK susceptibility relationship using xenogenic effector-target cell combinations. Our results suggest that NK lysis may not be influenced by enhanced MHC expression on xenogenic targets.

2. Materials and methods

2.1 *Tumour cells*

YAC (murine lymphoma) and K562 (human erythromyeloid leukemia) cell lines were propagated in culture in RPMI-1640 supplemented with 10% FCS, 2×10^{-5} M 2-mercaptoethanol, 300 µg/ml glutamine and 60 µg/ml gentamicin (complete medium).

2.2 *Effector cells*

Mouse and rat spleen cells were obtained by gentle teasing of spleens in complete medium as described before (Saxena and Adler 1979). Mononuclear cells from Rheses monkey peripheral blood were obtained by centrifugation over Ficoll-Hypaque gradients as described elsewhere (Saxena *et al* 1980). Spleen or peripheral blood derived mononuclear cells were used as effector cells without further fractionation.

2.3 *Up-regulation of MHC class I antigens*

Natural killer-resistance inducing factor (NK-RIF) preparations were used for boosting the expression of class I MHC antigens on target cells (Saxena *et al* 1989, 1992). Starting materials for partial purification of mouse and human NK-RIF preparations were the culture supernatants from Concanavalin-A activated mouse spleen cells or human peripheral blood mononuclear cells, respectively. Cell preparations (5×10^6/ml in complete medium containing 2% instead of 10% FCS) were cultured with 5 µg/ml of Concanavalin-A, for 2 days and culture supernatants were obtained by centrifugation. NK-RIF preparations from both murine and human sources were isolated by the method described for rat NK-RIF (Saxena 1987; Saxena *et al* 1988). Briefly, culture supernatants were subjected to ammonium sulphate precipitation step (80% saturation). Precipitated material was pelleted, dissolved in water and dialyzed extensively for two days against 0·2 M glycine-HCl buffer pH 2·0, to deactivate gamma interferon. Resulting material was fractionated on Sephadex G-100 columns and the NK-RIF peak i.e., fractions which induced augmentation of MHC I antigen expression on YAC (for mouse NK-RIF) or K562 (for human NK-RIF) tumour cells, were pooled and concentrated on Amicon membrane filters with 5 kDa cut off point. NK-RIF preparations used in this study had no IFN activity as assessed by biological and immunoassays, in which NIH reference IFN preparation was used as standard.

2.4 *Staining tumour cells for MHC I antigens*

Control and NK-RIF treated tumour cells were incubated (10^5/tube for 20 min at 4°C), with 50 µl of hybridoma supernatants reactive to MHC I antigens. For YAC

cells, a mixture of supernatants from hybridoma HB-13 secreting anti-H-$2K^k$ antibody and hybridoma HB-102 secreting anti-H-$2D^d$ antibodies, was used whereas for K562 cells hybridoma secreting an antibody reactive to a nonpolymorphic determinant on class I HLA antigens (HB-95), was used. All hybridomas were obtained from ATCC. Cells were washed twice with PBS and were suspended in 0·1 ml of a 1 : 20 diluted rabbit anti mouse Ig-FITC antibody preparation. After a 15 min incubation at 4°C, cells were washed and fixed in paraformaldehyde. For background nonspecific staining of control or NK-RIF treated tumour cells, the first step of treatment with anti-MHC I antibody containing hybridoma supernatants was omitted, and the cells were treated with the second antibody-FITC preparation alone. Cells were analysed on a Beckton Dickinson FACScan analyser. Tumour cells treated with second antibody alone were first run on the flowcytometer to fix the fluorescence gate above which cells would be considered to have specific staining for MHC I antigens.

2.5 *Chromium release assay of cytotoxicity*

Labelling of tumour cells and chromium release assay of cytotoxicity was performed as described before (Sarin *et al* 1989).

3. Results

3.1 *Effect of NK-RIF preparations on MHC I antigen expression on YAC and K562 cells*

We have previously described a novel factor (NK-RIF), released by mitogen activated rat spleen cells, which induced a marked augmentation in MHC I antigen expression in YAC tumour cells and an attendant resistance to lysis by murine NK cells (Saxena *et al* 1988, 1991, 1992). NK-RIF preparations with similar biological properties have recently been partially purified from murine and human sources (Saxena *et al* 1991, 1992). Effects of mouse and human NK-RIF preparations on the MHC I antigen expression on YAC and K562 cells respectively, has been shown in figure 1. These results show that NK-RIF preparations induced a dose dependent increase in the expression of MHC I antigens on tumour cells.

3.2 *Susceptibility of control and NK-RIF treated target cells to lysis by allogenic and xenogenic NK cells*

Lysis of control and NK-RIF treated YAC cells by mouse and rat NK cells was examined at several effector to target cell (E/T) ratios. Results of two representative experiments are shown in figure 2. A significant decline in the NK susceptibility of YAC cells was seen if mouse spleen cells were used as effectors. Control as well as NK-RIF treated YAC cells were however killed equally well by rat spleen effector cells. Similar experiments were done with control (MHC I negative) and human NK-RIF treated K562 target cells. Results of two representative experiments given in figure 3 clearly show that NK-RIF treated K562 cells were lysed poorly by human NK cells. NK-RIF treated K562 cells were not found to have acquired any resistance to lysis by NK cells in monkey peripheral blood. In fact, NK-RIF treated K562 cells appeared to be lysed better by monkey NK cells.

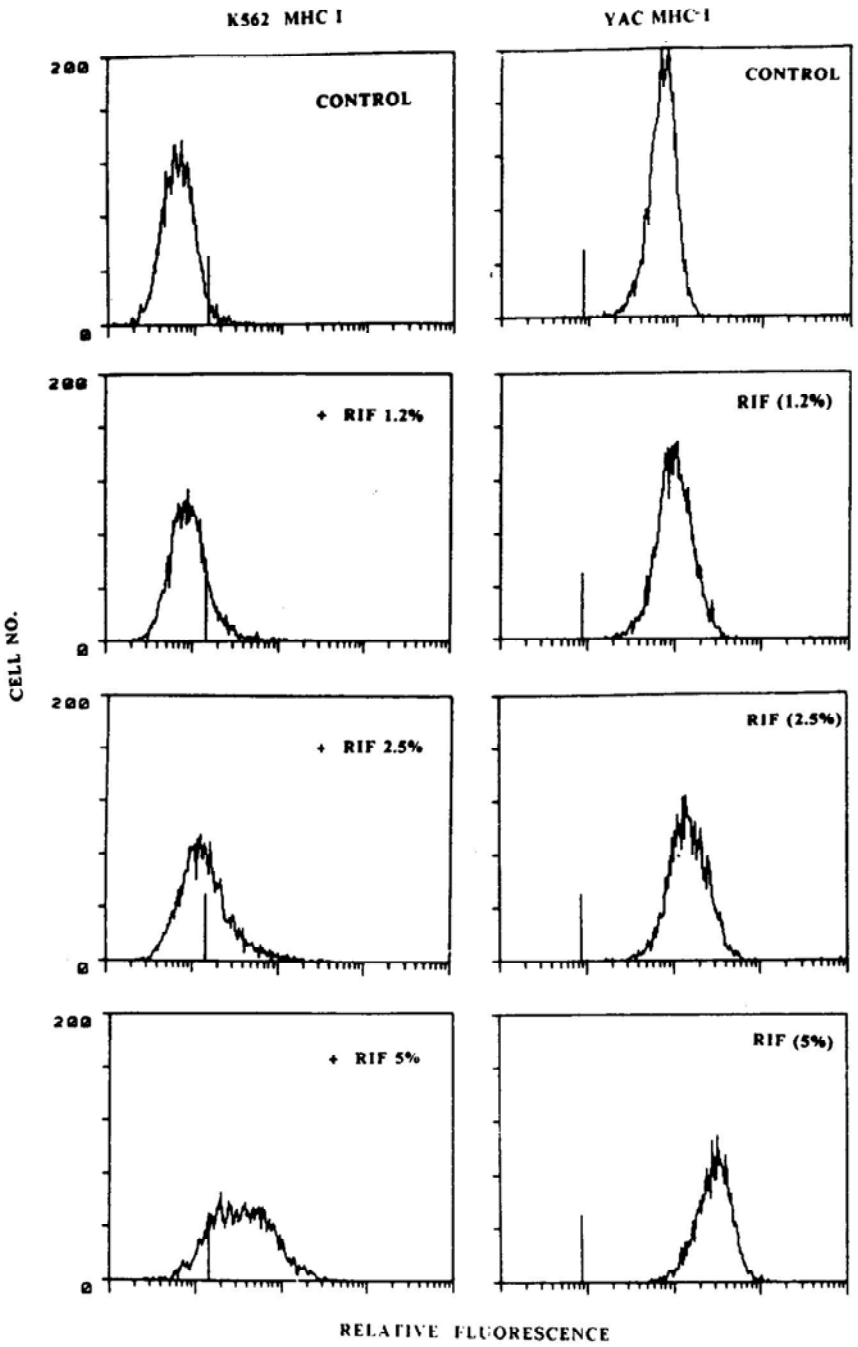

Figure 1. Effect of NK-RIF preparations on the expression of MHC class I antigens on K562 and YAC tumour cell lines. Tumour cells were seeded at a concentration of 5×10^4/ml in complete medium, with various given concentrations of NK-RIF (abbreviated as RIF in figure), ranging from 0 to 5% V/V. Human NK-RIF was used with K562 cell line and mouse NK-RIF was used with YAC cells. After two days of culture, tumour cells were washed and stained for class I MHC antigens by the procedure given in methods. Results of flowcytometric analysis of cells are given. Vertical line in each panel represents the gate set-with cells stained with second antibody only (non specific stain).

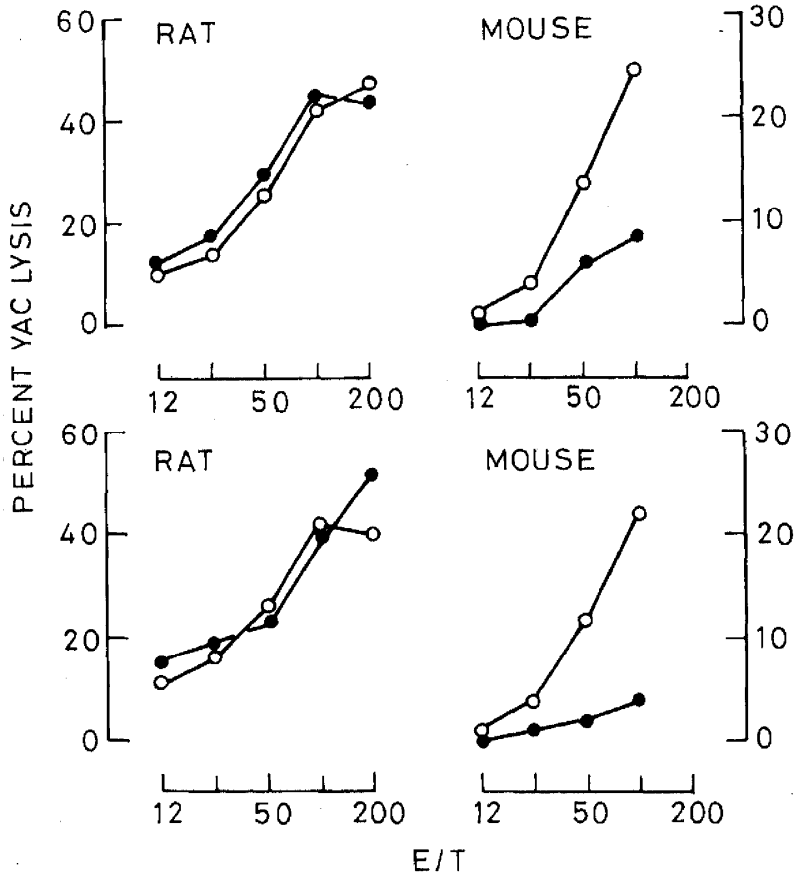

Figure 2. Lysis of control and NK-RIF treated YAC cells to rat and mouse NK cells. YAC tumour cells were cultured with (●) or without (O) 5% V/V of a mouse NK-RIF preparation (representing a final concentration of l0μg protein/ml) for two days. Cells were washed, labelled with chromium and used as targets in a 4h assay of cytotoxicity using mouse or rat spleen cells as effectors. Results of two experiments are shown (top and bottom panels). Each point represents mean lysis obtained in three replicate assay wells.

4. Discussion

For K562 and YAC tumour cells, an inverse correlation between class I MHC expression levels and susceptibility to allogenic NK effector cells, is well established (Ljunggren and Karre 1990; Storkus and Dawson 1991). In the present study, we have demonstrated that enhanced levels of class I MHC antigens on these tumour cell lines lowers their susceptibility to allogenic but not to xenogenic NK effector cells. A soluble factor purified from the culture supernatants of activated mouse spleen cells or human peripheral blood mononuclear cells, which is known to be a potent agent for enhancing the expression of class I MHC antigens on tumour

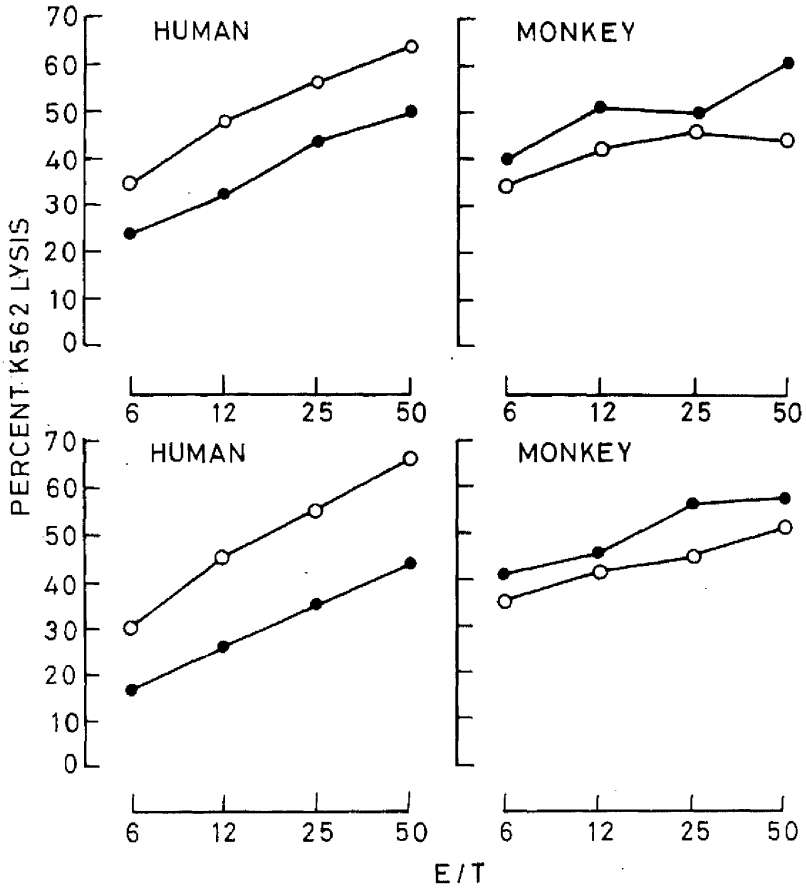

Figure 3. Lysis of control and NK-RIF treated K562 cells to human and monkey NK cells. K562 tumour cells were cultured with (●) or without (O) 5% V/V of a human NK-RIF preparation (representing a final concentration of 5 μg protein/ml) preparation for two days. Cells were washed, labelled with chromium and used as targets in a 4h assay of cytotoxicity, using human or monkey peripheral blood mononuclear cells as effectors. Results of two representative experiments have been shown (top and bottom panels). Each point represents mean lysis obtained in three replicate assay wells. Error bars have been omitted because variations amongst replicates were very low, being in absolute terms, less than 2% target lysis.

cell lines, was used for upregulating the expression of class I MHC antigens on these tumour cell lines. The factor called NK-RIF is a 12 kDa protein (rat NK-RIF), distinct from IFN and IL2, which induces NK but not CTL resistance in several tumour cell lines (Saxena *et al* 1988, 1992). NK-RIF treatment of tumour cells did not alter their ability to form conjugates with the effector cells (Saxena *et al* 1988).

While the mechanism of MHC I antigen related changes in NK susceptibility of target cells, is not clear, two hypotheses have been proposed to explain this

relationship (Ljunggren and Karre 1990). First hypothesis envisages a negative down-regulatory signal sent by class I MHC antigens to NK effector cells, and the second one postulates an interference by MHC I molecules in the target structure recognition by NK cells (Ljunggren and Karre 1990). The first hypothesis has received support from recent identification of a class of molecules (Ly 49 family) expressed on a subpopulation of NK effector cells, which may act as receptors for specific alleles of class I MHC antigens (e.g., D^d antigens for Ly 49 bearing subset of C57B1/6 NK cell), and appear to send an inhibitory signal to NK cells (Karlhofer *et al* 1992; Yokoyama and Seaman 1993). It should however be noted that Ly 49 mediated down regulation of NK cells is "global" in nature and D^d allele bearing targets are not lysed by Ly 49 bearing NK cells either directly or through ADCC, reverse-ADCC and LDCC routes. YAC cells which express D^d allele are however an exception to this rule (Yokoyama and Seaman 1993). In the human system, a new set of molecules (p58) expressed on NK cells have been identified which appear to have a role similar to Ly 49 antigens on murine NK cells (Moretta *et al* 1993). Human analogues of p58 however show no structural homology with the Ly 49 antigens (Moretta *et al* 1993). Molecules on NK cells which are specifically involved in down regulation of NK cell activity in response to MHC I alleles on YAC and K562 target cells, are not known at present. Moreover, our recent data suggests that the target interference model rather than the down-regulatory signal model, may explain the lower NK susceptibility of MHC I upregulated YAC and K562 cells (Haridas and Saxena 1995a,b). Irrespective of the mechanism of protection of MHC I upregulated YAC and K562 tumour cells, our results indicate that xenogenic effector NK cells do not appear to be sensitive to changes in the expression of MHC I antigens on the tumour cells used by us. This might be due to two possible reasons. Firstly, it is possible that the MHC I sensing molecules on the rat and monkey NK cells do not sense MHC I molecules expressed on mouse (YAC) and human (K562) target cells respectively. Alternatively, it is possible that allogenic and xenogenic NK effector cells recognize different target structures (TS) on YAC and K562 target cells and the TS recognized by allogenic but not the xenogenic NK cells, are subject to interference by target MHC I molecules. Further clarification of the mechanisms involved would require the identification of MHC sensing molecules on rat and monkey NK cells and the TS on YAC and K562 targets recognized by allogenic and xenogenic NK cells.

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