Understanding inverse correlation between the levels of class I major histocompatibility antigens on tumour cells and their susceptibility to lysis by natural killer cells: Evidence of competition experiments

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Natural killer (NK) cells, a class of lymphocytes distinct from T or B lymphocytes, can spontaneously lyse a variety of tumour cells without the need for a prior sensitization. A specific immune response against tumour cells results in generation of another class of cytotoxic effector cells, i.e. cytotoxic T-lymphocytes. Tumour cells must share some class I MHC antigens with the cytotoxic T-cell in order to be recognized and lysed by the latter. There is no such requirement for NK cells. Target cell MHC I antigen expression is however still an important factor in regulating the susceptibility of target cells to NK cells. An inverse correlation between the expression levels of class I MHC antigens on target cells and their susceptibility to NK cells has been found. In this article, I have evaluated the existing hypotheses for explaining the basis of inverse correlation between target MHC I expression and NK susceptibility, in view of results of our recent experiments in which the effect of competing tumour cells with normal or augmented MHC I expression, on the lysis of target tumour cells has been studied.

UNLIKE cytotoxic T cells, natural killer (NK) cells kill targets in a MHC (major histocompatibility complex) non-restricted manner. Class I MHC antigens on target tumour cells may, however, play a role in determining their NK susceptibility. An inverse correlation between the levels of class I MHC antigens on target cells and their NK susceptibility has been demonstrated in many studies[1–4], though exceptions have also been noted[5]. Ljunggren and Karre[1] proposed two possible mechanisms by which target cell MHC class I antigens may influence the NK susceptibility. According to the first model, MHC class I antigens on target cell membrane may interfere with the recognition of target structure molecules by NK effector cells. Nature of the target structures on tumour target cells is not clearly understood, though recent evidence seems to indicate that certain carbohydrate residues may act as target structures recognized by NK cells[6]. The second model for explaining the inverse correlation between the levels of class I MHC antigens on target cells and their NK susceptibility, proposes that class I MHC antigens on target cells, may send a down regulatory signal to effector NK cells.

If the activity of effector NK cells can be regulated by class I MHC antigens on the target cells, some receptor molecules on NK cells with target cell MHC class I molecules as their ligands, may be present. A family of type II integral membrane proteins, belonging to the superfamily of lectin-like molecules, has generated considerable interest in this regard. This family of molecules has, as its members, adhesion molecules of Selectin category (MEL 14/LAM 1, ELAM-1, etc.) which generally bind carbohydrate ligands in a calcium-dependent manner and are involved in directing the migration or homing patterns of leukocytes[7,8]. Ly 49 molecule, which is a homodimer of 84 kDa, belongs to this superfamily, and is expressed on a subset of murine (C57Bl/6) NK cells[9]. Available evidence indicates that specific class I MHC molecules ($H\text{-}2^d$ and $H\text{-}2^k$ molecules) on tumour target cells, act as ligands for Ly 49 molecules and this interaction sends a down regulatory signal to the effector NK cell, resulting in sparing of the target cell from NK cell mediated lysis[10,11]. In general, Ly 49^+ class of NK cells fail to lyse $H\text{-}2^d/H\text{-}2^k$-bearing targets, whereas Ly 49^- subset of NK cells may efficiently lyse the same target cells. A prominent exception to this rule appears to be YAC tumour cells which express D^d antigens but are efficiently lysed by both Ly 49^+ and Ly 49^- NK cells[9]. Since Ly 49 and/or related molecules may act as receptors for target cell MHC class I molecules, and send a down-regulatory signal to NK cells, it is tempting to hypothesize that this interaction may be responsible for the inverse correlation between the levels of class I MHC antigens expressed on

target cells and their susceptibility to NK cells. The problem with this explanation however is the global nature of inhibitory signal received through Ly-49 molecules. Ly 49 mediated turning off of the NK cells appear to be an 'all or none' phenomenon, since a target with the D^d molecule on its membrane is not lysed by Ly 49^+ NK cells either directly or through antibody dependent cell mediated cytotoxicity (ADCC) and reverse/redirected ADCC mechanisms[10,12]. Lysis of D^d bearing targets is possible by unfractionated C57Bl/6 NK cells. The latter represents a mixture of Ly 49^+ and Ly 49^- NK cells and as the Ly 49^+ effectors would be turned off by the D^d-bearing target cells, observed target lysis by the NK cell mixtures must be mediated by Ly 49^- effectors. Depressed susceptibility of D^d-bearing targets in which MHC class I antigen expression has been up-regulated by treatment with agents like interferon (IFN), can be demonstrated by using unfractionated C57Bl/6 NK cells[12,13]. It is obvious that the down regulation must operate on Ly 49^- NK cells. Therefore, there must clearly be other mechanisms not involving Ly 49 like molecules, responsible for the decline of NK susceptibility.

We have tried to understand the role of class I MHC molecules in determining the NK susceptibility of target cells by competition experiments in which NK effector cells are made to interact simultaneously with chromium labelled target cells and unlabelled competitor cells. In case competitor cells share target structures with the target cells, the former will competitively inhibit the lysis of the latter. NK cells would interact with target as well as competitor cells, and it can be reasonably assumed that any factor which selectively reduces the interaction between NK and competitor cells, will result in enhanced target lysis (Figure 1). This is similar to the case of enzymes, where reduction in the concentration of a competitive inhibitor results in an increased activity of the enzyme. The question we asked was whether modulation of class I MHC antigen levels on competitor cells results in changes in target lysis. We used a panel of five mouse and five human tumour cell lines and studied (a) their basal levels of class I MHC antigen expression and increase in response to IFN, (b) basal susceptibility to IL-2 activated NK cells and its modulation by IFN, and (c) their ability to compete in cold target inhibition assays and the effect of IFN on this competition ability[13–15]. Summary of all these results is given in Table 1. Each value in this table is a mean of 6–10 individual experiments. Mouse recombinant IFN-γ induced an increase in the expression of MHC I antigens on all murine tumour cell lines tested (top panel in Table 1). The increase ranged from a low of 36% for P815 cells to a high of 189.7% for YAC cells. In all cases except for EL4 target cells, there was a concomitant decline in the susceptibility of the target cells to IL2-activated NK cells. Effect of IFN treatment on the ability of the tumour cells to competitively inhibit the lysis of other tumour cells, or its own lysis, was also tested. All possible combinations of target and competitor cells were tested and the results have only been shown for the combinations where a significant competitive inhibition was observed. As shown in Table 1 (top panel), with the exception of EL4 tumour cells, interferon treatment reduced significantly the ability of tumour cells to compete with targets. Almost similar results were obtained

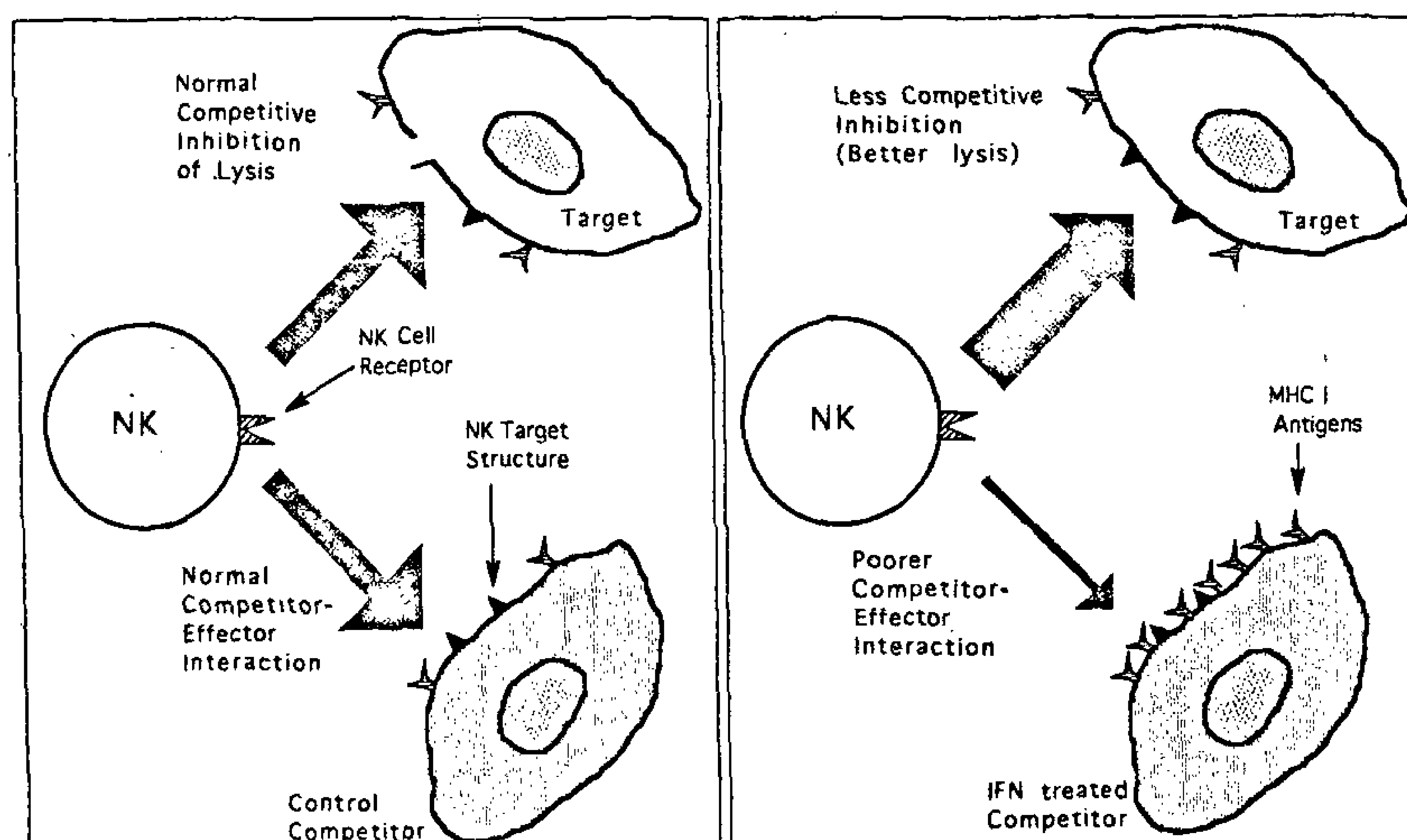

Figure 1. Left panel shows inhibition of NK cell mediated target lysis by a competing tumour cell. Presence of competitor cells with shared target structures, will engage NK cells and inhibit target lysis. In the right panel, MHC class I antigens on the competitor cell have been upregulated. Masking of target structures by MHC I antigens would lower the interaction between competitor and the NK cell, resulting in a better interaction with target cell. Inhibition of target lysis due to competition will be less and target lysis will improve as a result.

Table 1. Interferon induced changes in some tumour cell lines

Tumour cell line	Interferon induced change		
	Class I MHC level (% of control)[a]	IL2-NK susceptibility (% of control)[b]	Ability to compete (% decline in competition ability)[c]
YAC (H-2[a])	289.7	53.0	25 (YAC) 24 (P815) 36 (SP20)
P815 (H-2[d])	136.1	52.7	22 (YAC) 22 (P815) 27 (SP20)
SP20 (H-2[d])	178.6	56.0	23 (YAC) 23 (P815) 32 (SP20)
EL4 (H-2[b])	207.4	85.7*	9 (EL4)* 4 (L929)*
L929 (H-2[k])	177.7	58.5	25 (EL4) 12 (L929)*
K562	**	38.8	25 (K562) 28 (Molt 4) 43 (Raji) 32 (Daudi) 42 (HR7)
Molt-4	177.7	87.5*	5 (Molt-4)* 6 (Raji)* 3 (Daudi)* 3 (HR 7)*
Raji	106.6*	80.6	46 (Molt-4) 25 (Raji) 18 (Daudi) 41 (HR 7)
Daudi	Nil*	125.6	4 (Daudi)*
HR 7	182.5	66.6	18 (HR 7)

[a]Tumour cells were cultured at 0.2×10^6 cells/ml with and without 200 U/ml of murine (for mouse tumour cell lines) or human (for human tumour cell lines) IFN–γ at 37°C for 48 h. At the end of the incubation, cells were washed and MHC class I antigens were estimated by ELISA procedure described before[16]. Percent increase in the expression was calculated in each case and mean values from 6 to13 individual experiments for each tumour cell line, have been shown.

[b]Tumour cells were treated with IFN–γ as described above, washed and used as targets in a 4 h chromium release assay of cytotoxicity, at several E/T ratios. Lytic units/10^7 effector cells were calculated for control and IFN–γ treated target cells, and percent change in target susceptibility determined as described elsewhere[14,15]. Effector IL-2 activated NK cells were generated from mouse spleen cells or human peripheral blood mononuclear cells, by the method described before[14,15] Each value represents mean of 8–13 experiments.

[c]Tumour cell lines given in the first column were used as unlabelled competitor cells for targets shown in parantheses in the last column. Competition assays were done and percent decline in ability of tumour cells to compete as a result of IFN–γ treatment, were calculated as described before[14,15]. Each value represents a mean of 5–8 individual experiments.

*No significant change.

**MHC I antigens were induced on K562 cells which are otherwise MHC I negative.

nan tumour cell lines (Table 1, lower panel). It be noted that K562 cells had no basal expression s I MHC antigens, but the same could be induced tment with recombinant human IFN–γ. The value · cent increase in MHC I expression for K562 ; not given since the increase was from a zero e. In case of Daudi cells, there is a genetic defect prevents the production of MHC I molecules, uently IFN induced no increase in MHC I ex- n on Daudi cells. Except for MOLT4 and Daudi cells, in all other cases, IFN-treated tumour ce came less effective competitors as a result of IFN ment. In general it appears therefore (with excepti indicated above) that the IFN treatment induced levels of class I MHC antigens on tumour cells a associated with reduced NK susceptibilities and to compete in cold target inhibition assays.

Poorer competition by IFN-treated tumour cells ing in a better target lysis, is indicative of a \ competitor–effector interaction for IFN-treated co

tors. It is difficult to explain this finding by postulating a negative signal originating from elevated class I MHC antigen levels on IFN-treated competitor cells. Firstly, if down regulatory signals originating from IFN-treated competitor cells inhibit the effector NK cells non-specifically, one would expect a lower target lysis. Our results clearly show that target lysis improves if IFN-treated competitors are used, which argues against a non-specific turning off of the effector cells by exposure to cells bearing higher levels of MHC class I antigen. A second possibility is that the NK cells get specifically turned off against those tumour cells which send the inhibitory signal originating from elevated expression of MHC I antigens. In this case, IFN treatment of competitor cells should not influence target lysis unless one postulates a concomitant decline in the levels of target structures on IFN-treated competitor cells.

Of a total of 28 target/competitor combinations we examined, significant decline in the competitor's efficacy as a result of treatment with IFN, was observed in 20 cases. In the remaining 8 cases, no effect of IFN treatment on competition ability was seen. In no case did the use of IFN-treated competitor result in poorer target lysis. If MHC I molecules on tumour cells interfere with the recognition of competing tumour cells by NK cells (Figure 1), our results may be readily explained. Our results with competition experiments thus appear to support the target interference model rather than the negative signal model for explaining the role of MHC I antigens in regulating the NK effector–target interaction. As discussed above, negative signal hypothesis has found support with the demonstration of NK cell receptors for MHC I molecules on target cells. In our opinion, the two models discussed herein may not be mutually exclusive and which of the two mechanisms operates may depend upon the specific effector–target combination under study. A target cell which expresses MHC I antigens and is still susceptible to NK cells, is clearly not able to send a negative signal to NK cells. In such cases, if enhanced MHC I expression results in decline in NK susceptibility of the tumour cell, this decline may be due to interference by MHC I molecules, for which we have provided evidence here. On the other hand, if a specific receptor for target cell MHC I molecules is present on effector NK cells, and is able to send an inhibitory signal to the NK cells, the lack of lysis of such a target cell may be explained by the negative signal hypothesis.

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