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Natural Killer Cells and Their Targets: Impact of Differentiation on Target Cell Susceptibility*

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A. Introduction

Natural killer (NK) cells display selective binding/lytic properties insofar that they kill only certain target cell types. Although the recognition structures of NK cells and their respective targets are still largely unknown, accumulated data indicate that target susceptibility may be determined in part by the stage of differentiation of the target cells [1–3]. Thus, natural killer cells can frequently easily kill cells, both malignant as well as normal cells, at earlier "primitive" stages of differentiation. We have used this approach in testing cloned tumor cell lines undergoing controlled differentiation as an assay system for exploring the differentiation-related events in more detail. The present article summarizes our present state of knowledge as achieved by these studies.

B. Material and Methods

Basically our approach was to use a ^{51}Cr-release assay using various target cells, notably in vitro growing tumor cell lines as described previously [4]. Differentiation was induced under varying conditions and with widely different agents, and the consequences of differentiation were analyzed by a variety of markers including NK susceptibility. Changes in resistance to NK effector cells in the respective targets were in several cases also tested in relation to changes in the ability of the targets to function in cold target inhibition assays [5]. In all the differentiation systems studied the consequences of induction of differentiation on kinetics were followed, and any direct impact of the inducing agent on NK susceptibility of the targets was excluded.

C. Results

I. Tumor Systems Where Differentiation Leads to Resistance to NK Lysis

In our initial studies a series of tumor systems studied were all found to express an increase in NK resistance in parallel to the induced differentiation [1]. This was regardless of agent(s) used to induce differentiation. In most systems the resistance to lysis was accompanied by a parallel reduction in the capacity of the differentiated cells to function as cold target inhibitors in NK lysis. Our data obtained were thus compatible with the notion that NK cells tended to react against cells in the earlier stages of differentiation. Cells at a later stage of differentiation would be relatively deficient in their ability to react with NK cells, this probably accounting for a major (total?) part of the increase in NK resistance. The tumor systems studied included widely different types of cells such as teratocarcinomas, histiocytomas, and myeloid leukemias. A summary of the results obtained is shown in Table 1. In some of the tumor systems it was possible to obtain clones of cells of "spontaneous" origin with markers indicating increased differentiation. Such clones obtained without any

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Table 1. Tumor systems where induced differentiation leads to a decrease in NK susceptibility

Cell type	Cell line	Inducer	Differentiation markers	NK sensitivity
Erythroid leukemia (Human)	K562	Sodium butyrate or hemin	Glycophorin, hemoglobin	Decreased
Histiocytoma (Human)	U937	TPA	Fc receptors, HLA-DR	Decreased
Erythroid leukemia (Mouse)	GM-86	HBMA	Hemoglobin, surface proteins	Decreased
Embryonic carcinoma (Mouse)	PC13	Retinoic acid	Endodermal differentiation	Decreased

known added inducing agent displayed the expected decrease in NK susceptibility, as shown in Table 2. This showed therefore that the decrease in NK sensitivity indeed seemed to be linked to differentiation stages regardless of how this stage was reached/induced in the particular tumor under scrutiny.

II. Tumor Systems Where Differentiation Leads to Susceptibility To NK Lysis

Subsequent to the above studies we started to encounter tumor systems where induction to differentiation led to the opposite effects, namely an increase in NK susceptibility [3]. This was encountered in two systems, namely neuroblastoma cells in vitro and also when inducing differentiation in CLL cells freshly obtained from patients. Table 3 summarizes the results obtained in these two systems with regard to surface and morphological markers in relation to changes in NK susceptibility. It had previously been found that Burkitt lymphoma cells in general are more susceptible to NK lysis than the corresponding EBV-transformed B-cell lines [6]. The present studies thus add CLL cells to the category of cell types within the B-cell series that could be attacked by NK cells, in particular if induced to differentiate. In-

Table 2. Examples of "spontaneously" differentiating K562 cells expressing expected decrease in NK sensitivity

Cell type	Marker used for cloning	NK sensitivity
K562	–	50.0
K562, clone 4	Increased glycophorin	37.9 ($P > 0.025$)
K562, clone 6	Increased glycophorin	29.5 ($P > 0.001$)

Clones produced via agar cloning and screening for surface glycophorin-positive clones. Figures denote % specific release of ^{51}Cr mediated by NK cells at 100 : 1 ratio

Table 3. Tumor systems where induced differentiation leads to an increase in NK sensitivity

Cell type	Cell line	Inducer	Differentiation markers	NK sensitivity
Neuroblastoma	SH-SY5Y	TPA	Catecholeamines Nerve-specific enolase	Increased
CLL	Fresh cells	TPA	Cytoplasmic Ig	Increased

Table 4. Positive correlation between ability of TPA to induce NK susceptibility in CLL cells and active stage of disease

		Progressive disease	
		Yes	No
Increase in NK sensitivity[a]	Yes	14	2
	No	1	14

[a] Yes statistically increased NK susceptibility after TPA treatment. Figures denote number of CLL patients studied

terestingly, the ability of CLL cells to undergo differentiation leading to an increase in cytoplasmatic Ig and NK susceptibility could be shown to be positively correlated with the disease being in an active stage [3]. This correlation was found highly significant, as indicated in Table 4, where the aility to become NK susceptible could even be used as an indicator of imminent onset of active disease in patients with stable CLL disease.

D. Discussion

In the present article we have briefly summarized our evidence that NK cells may be able to bind and lyse certain targets depending at what stage of differentiation that particular cell is located at the time of the assay. The picture has some general features but also some quite sizeable controversial and complicated aspects. Thus, it is clear that NK cells may indeed have a general tendency to be aggressive against more undifferentiated cells within a cell lineage [1–3]. However, within a particular cell lineage sizeable variation may exist between cells at different stages of differentiation. Whereas bone marrow stem cells may belong to the NK susceptible "pool" [7] lymphoid cells of B-cell nature may or may not be susceptible. Thus, EBV lymphoblastoid cells are in general fairly resistant to NK lysis like most freshly obtained CLL cells [3]. Also, both NK-resistant as well as NK-susceptible cells may be found within human myeloma cells/cell lines, that is using cells supposedly very highly differentiated within this lineage [6]. One would then have to conclude that the NK susceptible cells within the B-cell lineage (mostly Burkitt-lymphoma cell lines, TPA-induced CLL cells from patients in active disease, and myeloma cells from certain patients) do not follow a safe and steady change in one direction only with regard to NK sensitivity upon differentiation. This could either be explained on the assumption that certain differentiation markers on B cells that can serve as NK target moieties may be able to express themselves at more than one time during differentiation of B cells. Alternatively, there may be some surface changes linked to differentiation which in more general terms may allow a cell type to change in a + or – fashion with regard to NK sensitivity. In particular this would seem to be the case for certain glycolipid changes where so far in the presently studied differentiation systems there has always been a positive correlation found between the change in concentration of these glycolipids (and their degree of sialic acid conjugation) and the corresponding changes in NK susceptibility [8]. The same differentiation-inducing agent, e.g., TPA, could be shown here to have an opposite impact on glycolipid composition in the two respective tumor groups (=displaying increase or decrease in NK sensitivity upon differentiation). Further analysis would be required to analyze whether these glycolipid changes cause the observed changes in susceptibility to NK lysis or whether they are merely a side phenomenon of no direct relevance.

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