Haematology and Blood Transfusion Vol. 28
Modern Trends in Human Leukemia V
Edited by Neth, Gallo, Greaves, Moore, Winkler

Structure and Function of the Transferrin Receptor – A Possible Role in the Recognition of Natural Killer Cells

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The monoclonal antibody OKT9 reacts specifically with the receptors for transferrin in human cells and has been used to isolate and characterise this receptor [1]. The receptor is a dimeric glycoprotein (Mr = 180,000) composed of two apparently identical subunits (Mr = 90,000) which are disulphide linked. The transferrin receptor appears to be a transmembrane molecule and is phosphorylated, the phosphate group being predominantly on serine residues. The cell surface form of the molecule possesses both complex and high mannose oligosaccharide chains, which do not appear to have a direct role in antibody (OKT9) binding. The molecule can be cleaved from the cell surface into a 70,000 molecular weight fragment, suggesting that the major part of the receptor is exposed to the extracellular environment. The released 70,000 molecular weight fragments are not disulphide linked and possess antibody (OKT9) binding sites. Cross-linking studies using radiolabelled transferrin suggest that two molecules of transferrin are bound to each 180,000 molecular weight receptor dimer. In addition, each 70,000 molecular weight fragment can independently bind one molecule of transferrin (Fig. 1).

We have observed that normal or malignant cells generally regarded as sensitive targets for so-called NK cells [2], e.g. cer-

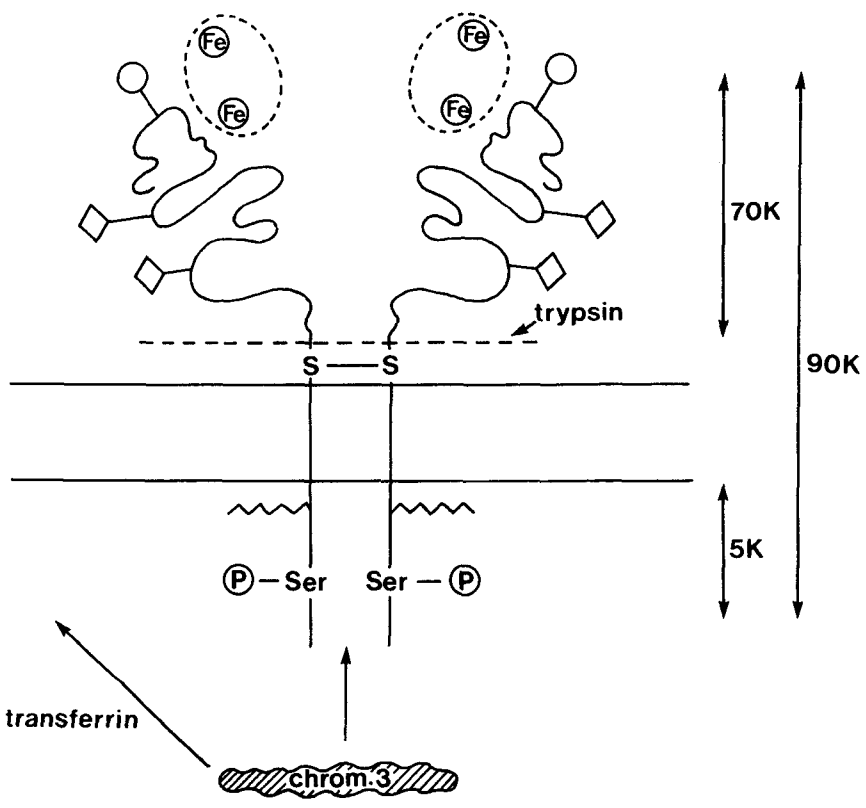

Fig. 1. Independent binding of the 70,000 molecular weight fragment to one molecule of transferrin. See [4] and [5] for details

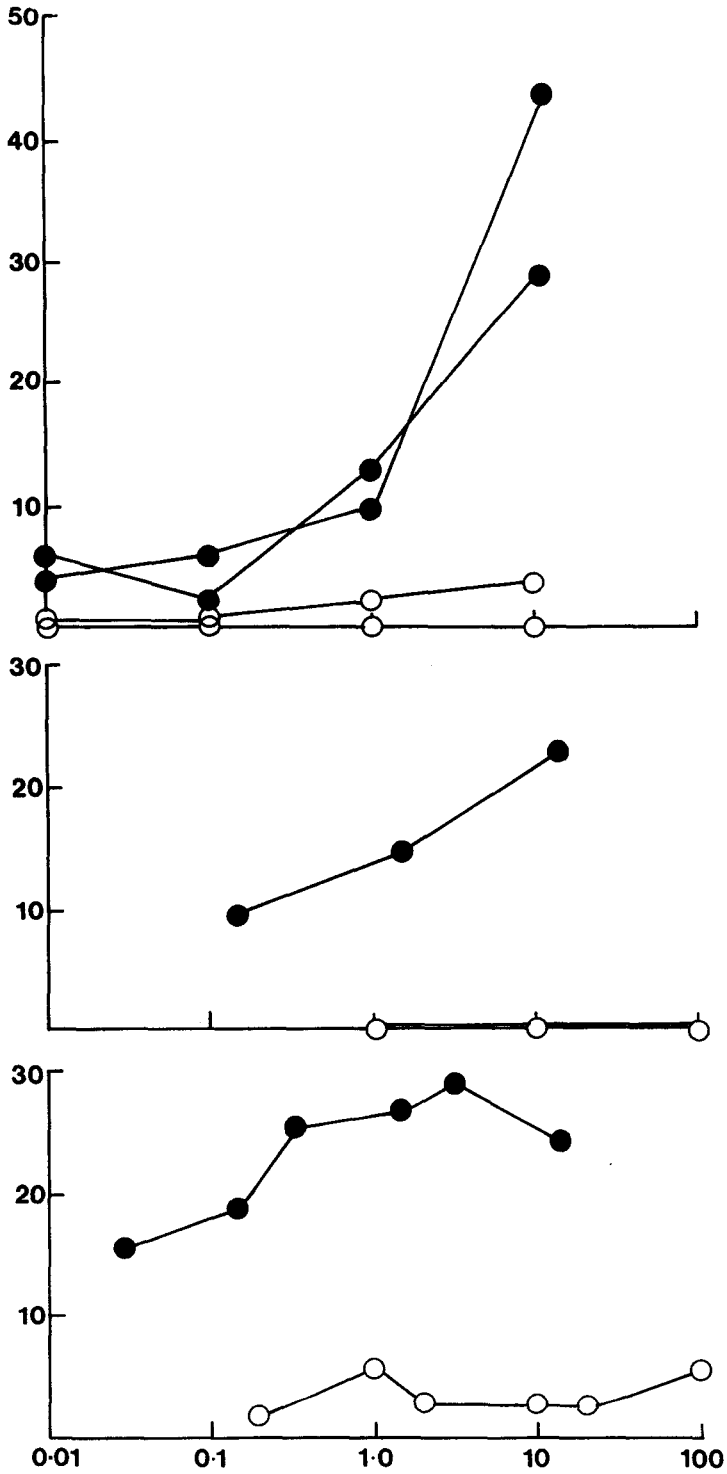

Fig. 2. Inhibition of NK activity by the purified trf receptor or purified HLA-AB molecules. *Vertical axis,* percentage of inhibition of ^{51}Cr release from K562 target cells; *horizontal axis,* concentration of trf receptor or HLA-AB molecules (μg/ml) in the assay mixture. O——O, HLA-AB; ●——●, trf receptor. Experiments 1A and 1B were done using the first batch of trf receptor and HLA-AB antigen on two separate occasions. E : T ratio = 100 : 1. Experiments 2 and 3 were done using the second batch of trf receptor and HLA-AB antigen. Experiment 2, E : T = 100 : 1; Experiment 3, E : T = 25 : 1

tain leukaemic cell lines such as K562 and T leukaemic lines, fetal thymocytes, haemopoietic progenitor cells, had a strong expression of transferrin receptors, whereas cell types that are found to be insensitive to NK attack, e.g. resting lymphocytes, some B-cell lines, have low or negligible transferrin receptor levels. This prompted us to investigate the possibility that trf receptors represent recognition structures for NK cells. Three approaches were adopted: (a) analysis of the correlation between sensitivity to natural killing and the proportion of trf receptor positive cells in different cell lines; (b) a study of the relationship between levels of trf receptor expression in cell lines and their capacity to inhibit, competitively, recognition and killing of the target cell K562 by NK cells and (c) a comparison of affinity purified, soluble, proteolytic fragments of trf receptor and HLA-AB molecules for their ability to inhibit the natural killing effect.

In initial experiments we tested human malignant and non-malignant cell lines for their sensitivity to killing by fresh human

mononuclear cells from peripheral blood, using the 4-h cytotoxicity assay according to Herberman and Holden [2]. The same target cells were tested for their expression of trf receptors using the monoclonal antibody OKT9, which was reacted with goat anti-mouse FITC ($F(ab')_2$). A pattern of NK sensitivity emerged, which divided those cell lines which had a high proportion of trf receptor positive cells into three categories: NK sensitive (40%–55% lysis), NK weakly sensitive (10%–40% lysis) and NK insensitive (1%–10% lysis). Those cell lines which weakly or negligibly expressed trf receptors were minimally sensitive or, mainly, resistant to NK cells.

Specificity of the recognition by NK cells was investigated by 'cold' target inhibition assay [3]. The capacity of test cell lines to compete with the ^{51}Cr-labelled K562 cell as targets for NK effectors and thus cause inhibition of ^{51}Cr release from K562 was compared with the competitive capacity of unlabelled K562 cells. The same batches of cells were tested for the presence of trf receptor using OKT9 and I^{125} goat anti-mouse Ig as a second layer antibody. In attempting to correlate inhibitory capacity of cells with trf receptor expression, we took the inhibitory capacity of 10^6 test cells expressed as a proportion of the activity detected with the same number of K562 inhibitory cells. Linear regression analysis indicated a direct correlation between the two parameters ($r=0.86$, $P<0.0005$) for cell lines having fewer trf receptors than K562. Significantly, however, cell lines with a higher total content of trf receptors than K562 did not express a greater inhibition capacity, giving an overall linear correlation with a corresponding $r=0.698$ ($P<0.0005$). This suggests that K562 could have some other advantage (for example, size, morphology, charge, hydrophobicity) as a competitor with itself in the assay.

Definitive involvement of trf receptors in NK recognition was tested by blocking the NK-mediated lysis of K562 cells with a 70K fragment (trypsin cleaved) of the receptor. A 38K HLA-AB fragment (papain cleaved) was used as control. The results from four similar experiments are shown in Fig. 2. The trf fragment partially inhibited the NK-induced lysis of K562 cells, whilst no significant inhibition was observed in samples incubated with the HLA-AB antigen even when the concentration of the HLA-AB molecules was ten times higher than the concentration of the trf receptor.

These experiments provide data compatible with our hypothesis. Thus, the existence of target recognition structures and the trf receptors are correlated. In addition, purified trf receptors could inhibit NK cytotoxicity. Incomplete inhibition may be due to the large degree of denaturation of the fragments, their rapid internalisation by the cells in the assay, lower avidity and/or the presence of additional target recognition structures on K562 cells.

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