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Daniel H. Rich*

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Adhesion Molecules as Drug Targets. The Case of CD2 [1]

Ellis L. Reinherz*

The cell surface protein CD2 was originally described as the receptor responsible for rosetting of T lymphocytes (T cells) with Sheep Red Blood Cell (SRBC). The ligand on SRBC is CD58 which is also present on B cells and antigen-presenting cells (APC). Beside the low affinity adhesion properties of CD2, the interaction of CD2 on T cells with APC is of major importance also for the activation of T cells by delivering secondary signals (in addition to the first signal by the T-cell receptor) which are essential for complete stimulation of the T cell. Once nonresponsiveness or anergy of a T cell has been induced, *e.g.* by blockade of the most important costimulatory interaction of CD28 (T cell) with B7 (APC), additional blockade of CD2 would prolong the anergic T-cell state even in presence of normally restoring exogenous interleukin-2 (IL-2). These data suffice to encourage pharma research for drug-discovery programs for blockade of CD2-CD58 interaction.

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CD2 belongs to the superfamily of immunoglobulin-like molecules and consits of two extracellular domains and a cytoplasmic tail which is very rich in prolines. Many signalling proteins bind to the cytoplasmic tail including the IL-12-responsive element sequence despite the lack of tyrosines in these sequences which are known to be involved in binding motifs. The two extracellular domains of CD2 are conjugated to a glycan-sugar moiety which lies apart from the CD58 binding site, but absence of the sugar after deglycosylation does inhibit binding of CD2 to CD58.

In addition to the CD58 binding site on CD2, an epitope has been defined by antibody binding studies – named CD2R – which is only found on the activated form of CD2. The epitope CD2R is dependent on the first protein domain but does not require the glycan domain (second domain). Using the method of amino-acid exchanges in mutated and re-expressed molecules, the binding domain could be defined for the CD2R-binding antibody. Cross-linking of two adjacent CD2 molecules is required for CD2R expression. Thus, the first step still remains the binding of CD58 to CD2 followed by expression of the CD2R epitope leading to a redistribution of all CD2 molecules at the site of contact of a T cell with a CD58 positive cell. It is remarkable that the whole process which happens within seconds works also in the absence of the CD2 cytoplasmic tail concluding that a particular intracellular signalling is not required. The whole process can be demonstrated by binding of the fluorescence-labelled antibody to clustered CD2- and, therefore, CD2R-positive T lymphocytes only, in contrast to other antibodies which bind also on nonactivated (and not aggregated) CD2 on T cells.

In conclusion although the interaction of CD2 with CD58 *per se* is only of low affinity type ($KD = 10^{-6}$) the copy number of adjacently clustered CD2 increases the interaction between CD2-positive T cells and CD58-positive cells and, therefore, enables stimulation of the T cell. With respect to design and discovery of new drugs, these findings might tell us that looking only at single isolated molecules, the absence of suitable sites for drug binding, and lack of drug discovery might not discourage from further attempts. However, new efforts should be initiated for drug discovery by looking at whole complexes and potentially newly assembled interaction sites of activated and, as shown here, clustered receptor surfaces with their respective ligands.

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