CD28 super-agonist T cell activation and the kinetic-segregation model

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Introduction

The correct explanation for leukocyte receptor activation must account for different ways in which activation can occur, such as with ligands or antibodies. CD28 is a transmembrane protein which co-stimulates the T cell receptor (TCR), leading to T cell activation. CD28 can also directly activate T cells in a TCR-independent way (Tacke et al., Eur. J. Immunol. 1997) unless T cells incubated with CD28 super-agonist in solution and dropped onto a surface coated with secondary antibodies proliferate and release IL-2. However, T cell activation is not observed for conventional antibodies with similar kinetic and binding constants. One difference between super-agonists and conventional antibodies is that super-agonists form planar complexes with receptors that are 75 Å shorter in height (Evens et al., Nat. Immunol. 2005 - Fig. 1), thus bringing the membrane in closer proximity to the surface.

Fig. 1: Structural analysis of antibody superagonism: superagonists bind membrane-proximal epitopes and form planar complexes. From Evans et al, 2005: crystal structure of a soluble form of CD28 (yellow) in complex with mitogenic 5.11A Fab (red), or non-mitogenic 7.386 Fab (green).

1. CD28 super-agonism requires immobilisation of cross-linked CD28 in close proximity with the surface

Fig. 2: Superagonism depends on antibody immobilisation. TCR-sufficient BW cells were transfected with mCD28, and incubated with a full superagonist (SA), fragment. Cells were then allowed to attach to glass surfaces coated with either anti-mouse Fc, anti-mouse heavy chain, or anti-mouse x-light chain secondary antibodies, or uncoated glass. IL-2 production was then measured.

Fig. 3: Superagonism depends on the valency of CD28. TCR-sufficient BW cells were stably transfected with mCD28, which forms a dimer, or with a FP1D1-mCD28 construct, which is monomeric. Cells were stimulated with the superagonist (SA) specific to their CD28 construct alone or in combination with an additional antibody that binds a different epitope. IL-2 production was then measured.

Fig. 4: Superagonism depends on the dimensions of the CD28 extracellular domain. (a) TCR-sufficient BW 5147 mouse leukemia cells were stably transfected with mCD28, or a hFc-mCD28 construct whose extracellular domain is lengthened by 75 Å. Cells were stimulated with anti-CD28 or anti-Fc superagonist (SA). (b) IL-2 production was measured.

2. Towards a molecular mechanism of CD28 super-agonism

Fig 5: Superagonists induce clustering of signalling sufficient CD28. TCR deficient BW cells were stably transfected with a mCD28-mEs3.2 fusion protein. Cells were stimulated with (a) no antibody (no ab) or (b) superagonist (SA) or (c) conventional antibody (conv ab) for 15 minutes and then applied to a cover slip coated with anti-Fc secondary antibody. PALM images were collected in TRF mode. Images display single representative cells. Scale bars: 2 µm.

3. A kinetic-segregation model for CD28 super-agonism

Fig 6: Superagonists slow down the diffusion of unligated, signalling deficient receptors. TCR deficient BW 5147 cells were stably transfected with a truncated mCD28-mEs3.2 fusion protein that lacks the cytoplasmic signalling domain. Cells were stimulated and imaged as described above (Fig 5). Trajectories were calculated by relating point-spread functions within 300 nm from one frame to the next (with a memory of 4 frames) (a-c). Jump distance (JD) distributions (d). A model with two diffusive populations was fitted to each JD distribution, revealing two different behaviours: (a) a very slow, approximately stationary population, and a moderately fast population. We interpret the slow population as antibody-ligated and the faster one as unligated CD28.

Fig 7: Kinetic-segregation model-based interpretation of antibody superagonism. The surface-immobilised superagonist (SA) binds a membrane-proximal epitope of CD28 and pulls the cell membrane and surface into closer proximity. Phosphatases with large ectodomains, such as CD45, are excluded very efficiently from regions surrounding antibody-bound-CD28. In the exclusion zones, kinases such as Lck dominate over phosphatases, favouring receptor triggering and signalling. Other receptors, such as the T-cell receptor (TCR), become trapped in exclusion zones by crosslinked CD28. They are ligand-independently triggered. Conventional antibodies (Conv ab), despite similar ability to crosslink CD28, exclude phosphatases less efficiently because the gap between cell membrane and surface is 75 Å larger. Other receptors are also trapped to a smaller extent. Net phosphorylation is lower and an additional stimulus is needed for activation.