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): resting 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 (Evans et al., Nat. Immunol. 2005 - Fig. 1), thus bringing the membrane in closer proximity to the surface.

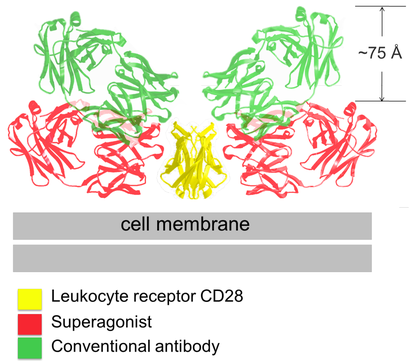


Fig. 1: Structural analysis of antibody superagonism: superagonists bind membrane-proximal epitopes and form planar complexes. From Evans et al, 2005: crystal structure of a soluble form of CD28 (yellow) in complex with mitogenic 5.11A1 Fab (red), or non-mitogenic 7.3B6 Fab (green).

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

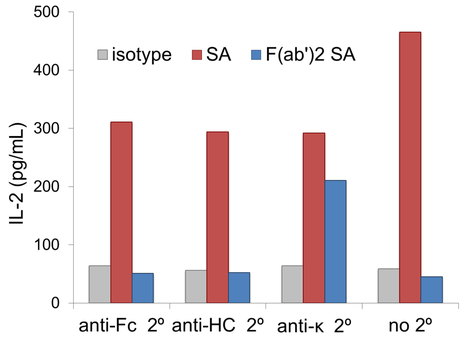


Fig. 2: Superagonism depends on antibody immobilisation. TCR-sufficient BW cells were transfected with rmCD28, and incubated with a full superagonist (SA) or with its $F(ab')_2$ fragment. Cells were then allowed to attach to glass surfaces coated with either anti-mouse Fc, anti-mouse heavy chain, or anti-mouse κ-light chain secondary antibodies, or uncoated glass. IL-2 production was then measured.

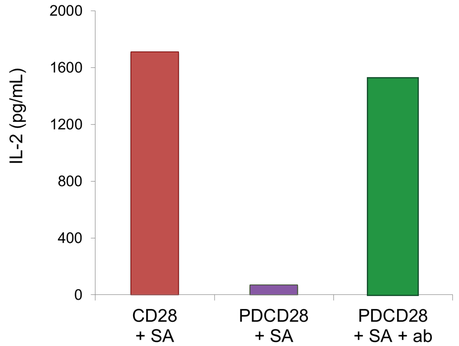
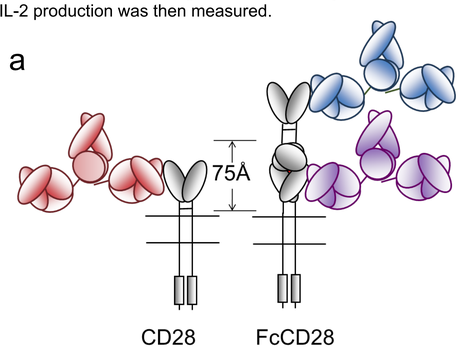


Fig 3: Superagonism depends on the valency of CD28. TCR-sufficient BW cells were stably transfected with rmCD28, which forms a dimer, or with a hPD1-mCD28 construct, which is monomeric. Cells were stimulated with the superagonist (SA) specific to their CD28 construct alone or in combination with an additional antibody that binds a different epitope. IL-2 production was then measured.



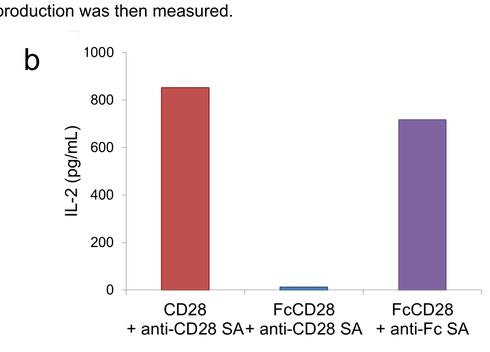


Fig 4: Superagonism depends on the dimensions of the CD28 extracellular domain. (a) TCR sufficient BW 5147 mouse leukemia cells were stably transfected with rmCD28, or a hFc-mCD28 construct whose extracellular domain is lengthened by 75Å. Cells were stimulated with anti-CD28 or anti-Fc superagonist (SA). **(b)** IL-2 production was measured.

2. Towards a molecular mechanism of CD28 super-agonism

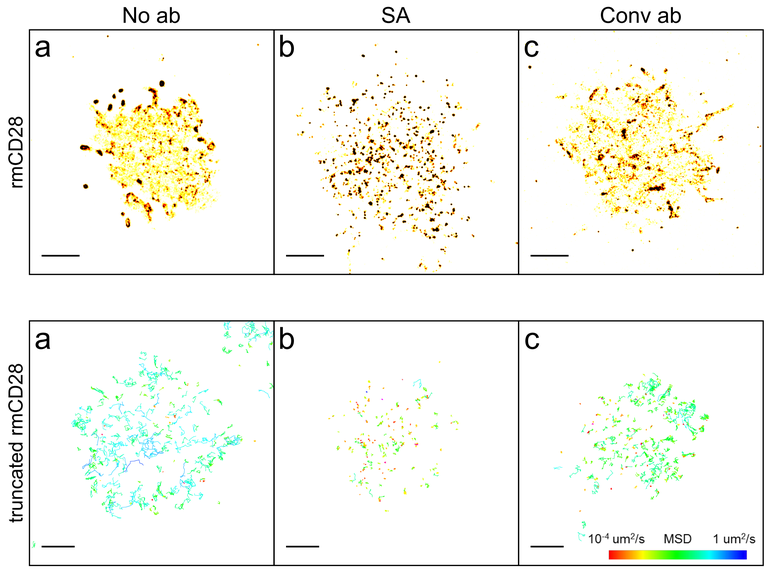
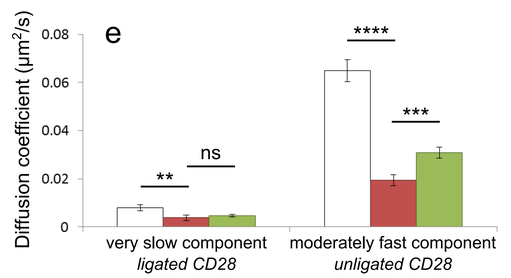


Fig 5: Superagonists induce clustering of signalling sufficient CD28. TCR deficient BW cells were stably transfected with a rmCD28-mEos3.2 fusion protein. Cells were stimulated with **(a)** no antibody (no ab) or **(b)** superagonist (SA) or **(c)** conventional antibody (conv ab) for 15 minutes and then applied to a cover slip coated with anti-Fc secondary antibody. PALM images were collected in TIRF mode. Images display single representative cells. Scale bars: 2 µm.

Fig 6: Superagonists slow down the diffusion of unligated, signalling deficient receptors. TCR deficient BW 5147 cells were stably transfected with a truncated rmCD28-mEos3.2 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 **(e)**: 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.



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

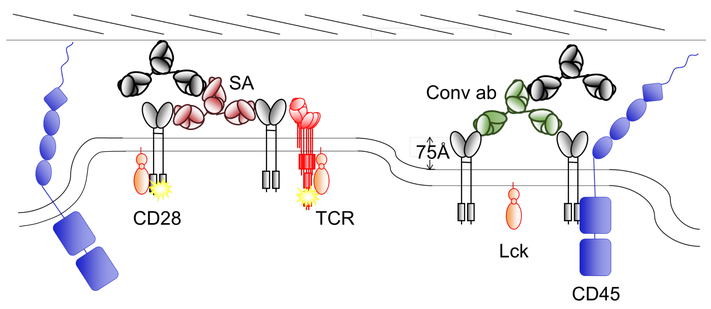


Fig. 7: Kinetic-segregation model-based interpretation of antibody superagonism. The surface-immobilised superagonist (SA) binds a membrane-proximal epitope of CD28 and pulls the cell membrane and surface into close proximity. Phosphatases with large ectodomains, such as CD45, are excluded very efficiently 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 where 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 75Å larger. Other receptors are also trapped to a smaller extent. Net phosphorylation is lower and an additional stimulus is needed for activation.