

MAb or SuperMAB?

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SuperMAB defines a class of superagonistic antibodies that activate T-cells independently of T-cell receptor engagement, and therefore have the potential to be a uniquely effective means of immune reconstitution and immunomodulation.

T-cells play a central role in initiating and terminating an immune response and are therefore ideal targets for immunotherapeutic intervention. Classical approaches to T-cell-mediated immunotherapy aim to strengthen the response of T-cells specific for microbial or tumor antigens. Examples are the expansion of T-cells by cytokines or T-cell vaccination with tumor antigen-specific peptides. Conventional immunotherapy of autoimmune/inflammatory diseases targets the physical or functional elimination of autoreactive T-cells or their products such as pro-inflammatory cytokines.

A novel approach using superagonistic monoclonal antibodies (SuperMABs) has recently been developed that balances T-cell activation and expansion by triggering co-stimulatory CD receptors without T-cell receptor engagement. SuperMABs convert co-stimulatory CD receptors into directly activating stimulatory CD receptors. This technology can be used to expand conventional immunocompetent T-cells and to activate immunomodulatory 'regulatory T-cells' which can prevent autoimmune diseases.

The SuperMAB technology

Activation and proliferation of the immune system's naive T-cells is normally only triggered in the presence of two signals: antigen carried by professional antigen presenting cells (APCs) has to interact with the T-cell antigen receptor (TCR) and a co-stimulatory ligand on the APC surface

must bind to a co-stimulatory receptor. CD28 is the most efficient CD receptor that co-stimulates naive T-cells in combination with the TCR. TeGenero has identified a functionally novel class of superagonistic monoclonal antibodies directed against CD28 which activates T-cells by just one signal - regardless of their TCR specificity - and thus has the advantage of bypassing the requirement for TCR triggering (Figure 1).

A different epitope

Conventional CD28-specific antibodies are co-stimulatory since they only activate T-cells in vitro when combined with a TCR stimulus. They differ from SuperMABs by

their inability to induce T-cell activation in vivo or in vitro when administered without TCR engagement. In the quest for the critical difference between co-stimulatory and superagonistic anti-CD28 antibodies, a binding site was identified in the extracellular domain of CD28 which is specifically recognized by SuperMAB specific for rat or human CD28, but not by conventional anti-CD28 antibodies.

According to a model of the human CD28 molecule, residues 60-65 form the superagonistic epitope, which is located between the C' and D strands at the 'edge' of the V-set domain. In contrast to the superagonists binding specifically to the C' D loop, conventional co-stimulatory CD28

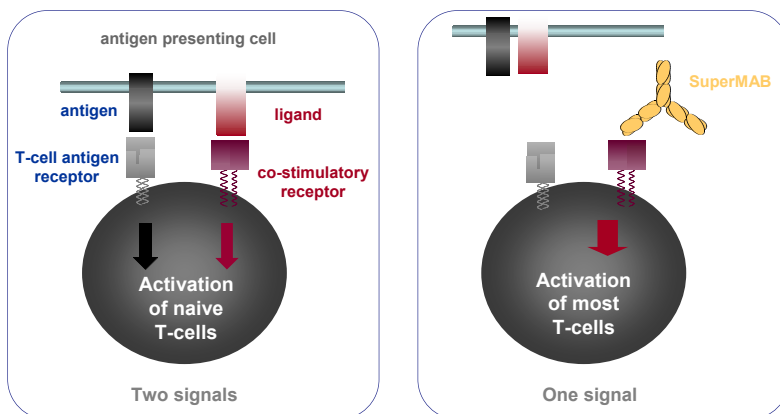


Figure 1. Activation of the CD28 signaling pathway normally requires simultaneous triggering of the TCR by antigen and of CD28 by its physiological membrane-bound ligands (left). CD28-SuperMAB bypasses the requirement for TCR signaling and activates T-cells in a single step (right).

monoclonal antibodies are critically dependent on the species-specific amino acid side chain at position 98, adjacent to the ligand binding MYPPPY motif (positions 99-104, Figure 2). Significantly, the superagonistic epitopes of rat and human CD28 are conserved.

Targeting immunodeficiency

Loss of T-cells due to chemo- or radiotherapy, HIV infection, hematopoietic tumors or lymphotoxic agents results in immunological deficiency that often causes severe opportunistic infections and tumors. Consequently, identification of a drug that effectively mediates regeneration of T-cell numbers and function in immunocompromised patients is one of today's major unmet medical needs. A well-tolerated T-cell stimulatory compound would also be an invaluable companion drug as an adjuvant for difficult vaccines such as those against tumors and some viruses, as well as for HIV flush-out therapy. Preclinical in vivo animal studies and work with cultured human T-cells has shown that CD28-SuperMAB effectively induces increased T-cell numbers and function.

No cytokine storm

Co-stimulation by CD28 not only induces cycling of TCR-stimulated cells, but also protects the expanding cells from apoptosis. Consequently, T-cell expansion by co-stimulation with artificial TCR and CD28 ligands could be a potent approach for polyclonal unbiased T-cell expansion. However, this is only feasible in vitro, when both the anti-TCR complex and conventional anti-CD28 are co-immobilized on artificial surfaces. In vivo, such strategies are hampered by the need to co-present the two antibodies in an immobilized form. Moreover, polyclonal activation of T-cells in vivo via TCR signals results in a burst of pro-inflammatory and pro-apoptotic cytokines known as the 'cytokine storm', and hence is not suitable for generalized stimulation of T-cell recovery. Thus, the need for TCR engagement has so far hampered the exploitation of the unique mitogenic potency of CD28 for the in vivo therapy of T lymphopenia.

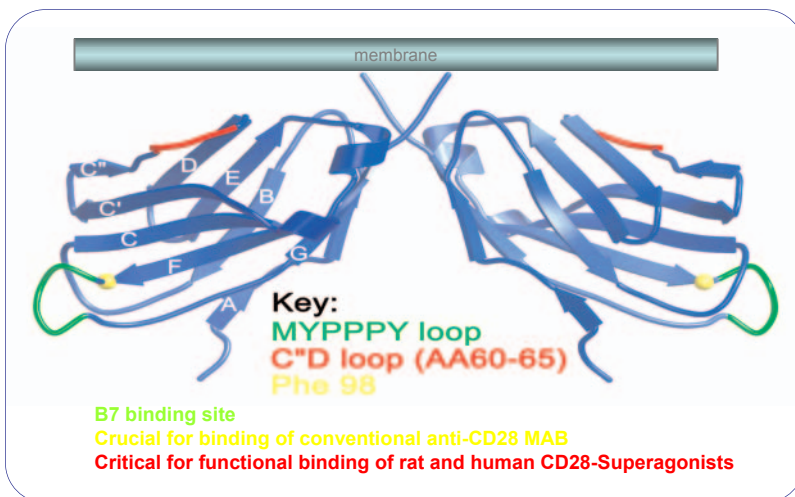


Figure 2. Depiction of the epitopes for superagonistic and conventional CD28-specific MAB in a 3D model of the extracellular part of human CD28. The MYPPPY motif (aa 90-104) critical for B7 binding is indicated in green, the adjacent aa 98 residue critical for binding of the conventional rat and mouse CD28-specific MAB is highlighted in yellow, and the C' D loop responsible for the binding of superagonistic rat and human CD28-specific MAB (aa 60-65) is indicated in red.

SuperMAB in lymphopenic rats

T-cell recovery in adult lymphopenic patients proceeds primarily from the periphery, takes months to years, and is characterized by oligoclonal expansions. Since antigen-driven proliferation is much more rapid than spontaneous, homeo-static recovery, an ideal therapeutic agent should promote expansion of all residual T-cells with the speed and efficiency of antigen-driven expansion, while leaving T-cell function intact for future immune responses. At least with regard to CD4 T-cells, CD28-SuperMAB fulfil these requirements.

In lethally irradiated bone marrow-reconstituted hosts, CD28-SuperMAB is able to dramatically accelerate repopulation by a small inoculum of mature T-cells. CD28-driven recovery of CD4 cells seems to be superior to that of CD8 T-cells. CD28-SuperMAB-expanded CD4 T-cells maintain repertoire diversity and are functional both in vitro and in vivo. This suggests that treatment with human CD28-SuperMAB should protect T lymphopenic patients from opportunistic infections.

The potent mitogenic effect of CD28-SuperMAB addresses T-cells of all

subclasses regardless of the antigen experience of their T-cell receptor. In addition, it was recently demonstrated that CD28-SuperMAB also prevents T lymphocyte apoptosis in rats by dampening expression of the apoptosis-inducer CD95L and promoting expression of the anti-apoptotic factor Bcl-xL. Together, the data demonstrate the unique potential of CD28-SuperMAB to act both as a T-cell survival and a T-cell growth factor in an intact organism.

Targeting autoimmunity

The T-cell expansion mediated by polyclonal CD28-SuperMAB in vivo is accompanied by the expression of anti-inflammatory cytokines, most notably of IL-10, rather than by the toxic cytokine storm of pro-inflammatory mediators induced by agents that address the TCR complex. Most importantly, CD28-SuperMAB over-proportionately expands regulatory T-cells, a specialized T-cell subset that suppresses auto-aggressive T-cells present in the body and which has only recently been appreciated as important guardians of immune tolerance. Based on their functional potency in suppression of organ-specific as well as

systemic autoimmune diseases, regulatory T-cells have been widely accepted as attractive targets for immunotherapeutic intervention. However, attempts to expand and activate this subset of CD4 T-cells in vivo and, ultimately, in autoimmune/inflammatory diseases have been hampered so far by the lack of therapeutic agents.

Therapy with CD28-SuperMAB

The pronounced in vivo expansion of regulatory T-cells induced by CD28-SuperMAB treatment in rats prompted TeGenero and co-workers to study the prophylactic and therapeutic efficacy of CD28-SuperMAB in established animal disease models for human autoimmune/inflammatory diseases. For example, in rat adjuvant arthritis, an established model for human rheumatoid arthritis, application of CD28-SuperMAB demonstrated a pronounced benefit in terms of both prevention and treatment, as determined by clinical scoring, ankle joint histology and weight loss scoring. In accordance with a key role for regulatory T-lymphocytes as mediators of CD28-SuperMAB-based anti-inflammatory function, the level of induction of

regulatory T-lymphocyte by CD28-SuperMAB was identical in arthritis-prone rats and healthy animals.

Experiments to determine the therapeutic potency of various formats of CD28-SuperMAB in diverse animal models for autoimmune/inflammatory diseases are ongoing. Preclinical results to date indicate that CD28-SuperMAB is capable of inhibiting the ongoing inflammation characteristic of RA (and possibly related diseases) in a well-tolerated fashion, thereby addressing the medical need in chronic autoimmune/inflammatory diseases, which require long-term therapy without severe side effects.

Outlook

The present findings suggest that CD28-SuperMAB provides a powerful therapeutic tool for the in vivo expansion of CD4 T-cells. The central importance of CD4 T-cells for the orchestration of the immune response suggests that if successful in humans, CD28-SuperMAB therapy may become a widely applicable and uniquely effective means of immune reconstitution.

Of note, CD28-SuperMAB overproportionally stimulates a subset of so-

called regulatory T-cells that control auto-aggressive T lymphocytes implicated in autoimmune diseases such as RA. The potential thus exists to develop CD28-SuperMAB antibodies against a wide range of T-cell-mediated autoimmune/inflammatory diseases, including RA, multiple sclerosis, psoriasis and potentially even diabetes.

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