TGN1412: Superagonist Dr. Jekyll and Superantigen Mr. Hyde

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INTRODUCTION

Last March, a phase I clinical trial resulted in severe adverse effects for the six volunteers receiving the experimental antibody TGN1412. As preliminary reports of the UK Medicines and Healthcare products Regulatory Agency have ruled out contamination of the antibody preparation as the source of the adverse reaction, the action of the antibody itself has been blamed. Since experimental work in animals did not show significant toxicity, progression to clinical testing ensued with the known results, and speculation has arisen as to how TGN1412 could trigger such response and as to whether it could have been predicted. Analysis of some features of TGN1412 structure and mechanism of action suggest that it could behave as a superantigen, and point to some cautions to be kept in the future when testing similar reagents.

MECHANISM OF ACTION OF CD28 SUPERAGONISTS

TGN1412 is a member of a class of monoclonal antibodies termed superagonists that target the T cell co-stimulatory molecule CD28. Treatment of T cells with anti-CD28 superagonistic antibodies overcomes the need for a primary TCR-dependent signal and results in T cell activation on its...
own, hence the term «superagonist»(1). They were originally described for rat CD28, and their activity has been linked to the generation of «signalling competent» CD28 oligomers by virtue of their binding to the C''D loop in the immunoglobulin variable region of the molecule, far from the binding site of the CD28 ligands B7.1 and B7.2(2) (Fig. 1). Such «signalling competent» CD28 promotes activation and nuclear translocation of transcription factors in the absence of overt proximal T cell signalling events characteristic of TCR-dependent T-cell activation, like ζ phosphorylation and ZAP-70 activation. However, superagonist CD28 antibodies require the TCR signalling machinery and promote the activation of Lck, LAT, PLCY1 and PKCtheta, leading to the suggestion that CD28 superagonists work by enhancing low level tonic signals from the TCR(3, 4).

In the rat system, preferential expansion of Th2 and Treg cells has been shown after in vivo treatment with anti-CD28 superagonistic antibodies(5-6). Furthermore, when tested in animal models of autoimmune Th1 diseases, like EAE and reactive arthritis, they showed a therapeutic effect(7,8). These promising results prompted the interest towards the potential benefits of similar reagents in humans. Subsequently, superagonistic antibodies to human CD28 were developed that confirmed the TCR-independent activation of nuclear factors, cytokine production and proliferation(2). TGN1412 was developed by humanisation of the Fc binding portion of one of these antibodies that made it suitable for human therapy(9). Paradoxically, the only therapeutic application of TGN1412 suggested so far is not of an immunosuppressive nature, since it has been reported to induce potent anti-tumour cytolytic activity in PBMCS from B cell chronic lymphocytic leukaemia affected patients(10), suggesting a predominant immunostimulatory activity.

LESSONS FROM THE THERAPEUTIC USE OF ANTI-CD3 AGONISTIC ANTIBODIES

T cells have long been the target of strategies aimed at preventing unwanted immune responses. In fact, OKT3 was the first monoclonal antibody used in the clinic, even before its target, the TCR-CD3 complex, was identified. OKT3 promoted immunosupression and prevented transplant rejection in kidney transplant recipients(11, 12). However, it also triggered an acute cytokine release syndrome that was prevented by corticosteroids(13). In addition, its therapeutic efficacy was limited by the development of neutralising antibodies(14). This could be overcome by humanisation of the Fc portion of the antibody. On the other hand, studies in mice showed that low affinity and non FcγR binding antibodies avoided the cytokine release syndrome whilst maintaining the immunosuppressive activity. Consequently, humanised non-FcγR binding versions of OKT3 and other anti-CD3 antibodies were developed(15, 16). The combination of both strategies has produced a safe and efficient therapy for transplant rejection and autoimmune syndromes. Interestingly, their mechanism of action is very similar to that reported for CD28 superagonists, as they also promote the development of regulatory T cells(17).

Several other factors can contribute to exacerbate the severity of the toxic syndrome in the case of the London volunteers in comparison to the early anti-CD3 clinical trials. Not the least important is the immune status of the recipients: the men in London were healthy individuals with an intact immune system while the participants in the kidney transplant trials were already under an immunosuppressive regime to prevent transplant rejection. Another important factor is the dose of antibody given: although the single dose of 100 µg/kg used in the case of TGN1412 is within the range used in the therapeutic protocols currently applied with the non-FcγR binding anti-CD3 antibodies, the latter is preceded by a dose-escalating regime starting in the ng/kg range.

In the development of TGN1412, consideration has been given to the humanisation side of the equation, but the
possibility of deleterious effect of agonistic antibody crosslinking on T cells has been ignored, despite what the history of therapeutic treatment with agonistic anti-CD3 antibodies clearly illustrates. In fact, humanisation probably improved FcγR binding that may have contributed to the toxicity of TGN1412.

AFFINITY AND THE CASE OF BACTERIAL SUPERANTIGENS

The likely cause of the adverse effects suffered by the volunteers during the clinical trial of TGN1412 is a cytokine release syndrome triggered by overstimulation of T cells, similar to that described in the initial trials of OKT3(18). Given that such an effect had not been reported previously either in the animal model studies of CD28 superagonists or in the preclinical testing of TGN1412 in rhesus and cynomolgus monkeys, differences in affinity at both ends of the antibody have been signalled as responsible. On the antigen-binding region, differences surrounding the antigen binding site between human and rhesus CD28 could result in reduced affinity of TGN1412 for rhesus CD28. Likewise, on the Fc-binding region the affinity of rhesus FcγR for humanised antibodies is likely to be lower than for endogenous antibodies. The combination of both circumstances would result in decreased interaction of the humanised antibody with rhesus CD28 and reduced T cell activation, masking a potential toxic effect in the human system. In the absence of experimental data to support the differences in affinity, this remains a working hypothesis.

The affinity hypothesis provides an interesting parallel between the behaviour of CD28 superagonist and bacterial superantigens in humans and animal models. Bacterial enterotoxins stimulate a large fraction of peripheral T cells in a TCR Vβ specific manner and are, therefore, termed superantigens(19). Just as suggested for TGN1412, bacterial superantigens trigger in humans a cytokine storm leading to a lethal toxic syndrome, mainly mediated by TNFα release. In contrast, in mice the response is milder and, in order to be lethal, they must be sensitised with galactosamine or LPS(20). Such differences in the magnitude of the response have been attributed to the affinity of the specific bacterial toxins for the TCR Vβ regions on the T cells and for the MHC-II on the APCs, since they are lower for both murine molecules than for their human counterparts(21). In addition, given that CD28 is expressed on all T cells, the response to TGN1412 is not Vβ-restricted but targets the whole T cell population.

The similarities in the behaviour of superagonistic antibodies and bacterial superantigens are not restricted to the affinity of their interaction with their ligands, but are manifested in the progression of the response that follows as well. As mentioned, the initial response to bacterial superantigens induces an acute release of cytokines. Although the toxicity is mainly mediated by TNFα and IFNγ, a full array of cytokines is produced. However, after the initial burst, a predominant Th2 and Treg phenotype emerges(22-29). Similarly, the reports for rat superagonistic antibodies showed that the predominance of Th2 and Treg cells is preceded by a general activation of T cells, including production of the prototypical Th1 cytokine, IFNγ(30).

In summary, the changes introduced to ensure the efficacy of superagonistic CD28 antibodies in humans may have maximised their potency to the extent of converting TGN1412 in a superantigen-like ligand with devastating acute effects.

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