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Perspective

T-Cell Costimulation — Biology, Therapeutic Potential, and Challenges

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The immune system has the remarkable ability to defend against diverse microbial pathogens and yet not to respond to self. T cells are key mediators of the immune response, and their activation

is tightly regulated to prevent autoreactivity. The processes of T-cell activation and self-tolerance are therefore potential targets for manipulation by drugs — hence, the recent phase 1 trial of a “superagonistic” monoclonal anti-CD28 antibody that was conducted in Britain on behalf of the German firm TeGenero, with unexpected and devastating results that are described in the report by Suntharalingam et al. in this issue of the *Journal*.

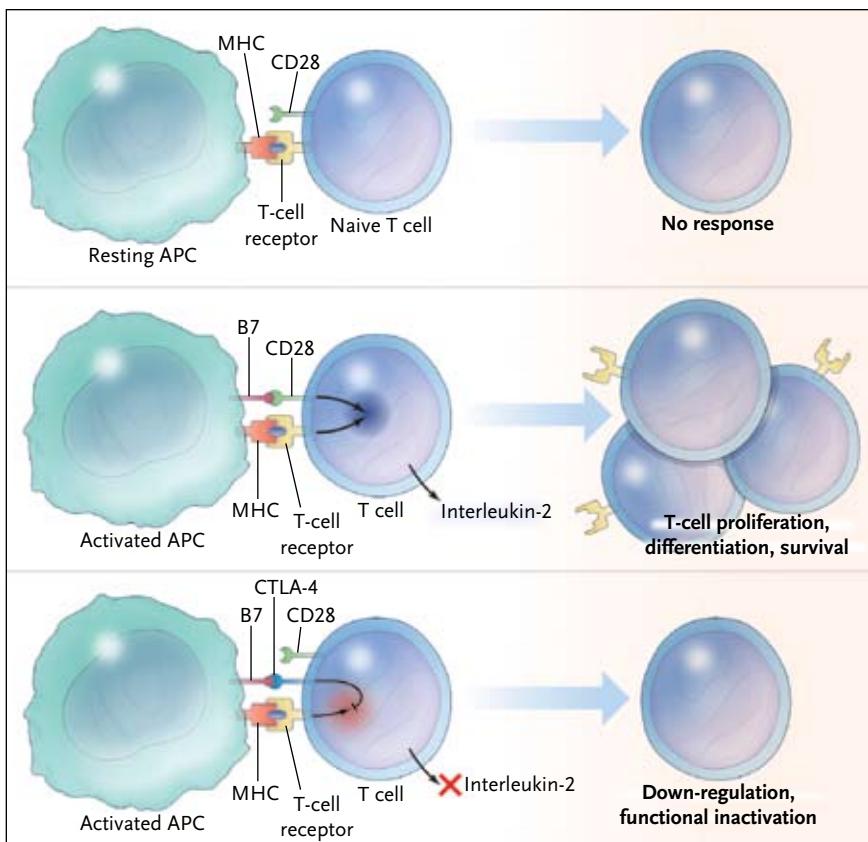
T-cell activation requires two signals that are delivered by antigen-presenting cells (APCs) (see figure). The first signal is antigen displayed by APCs in the form of peptides bound to histocompatibility molecules; the recognition

of antigen by T-cell receptors provides specificity to the response. The second signal, called the “costimulatory signal” because it stimulates T cells in conjunction with antigen, is provided by molecules on APCs that engage particular costimulatory receptors on T cells. In the absence of costimulation, T cells that recognize antigen either fail to respond and die or enter a state of unresponsiveness known as anergy. Thus, costimulation is a key determinant of the outcome of a T cell’s encounter with antigen.

The best-characterized T-cell costimulatory pathway involves the CD28 receptor, which binds to two costimulatory molecules, B7-1 (CD80) and B7-2 (CD86).¹

CD28 is constitutively expressed on all T cells in mice and on 95% of CD4+ T cells and 50% of CD8+ T cells in humans. B7-1 and B7-2 are expressed mainly on APCs, including dendritic cells, macrophages, and B cells. The expression of B7-1 and B7-2 on APCs is enhanced by the presence of microbes and by cytokines that are produced in response to microbes. This regulated expression of B7 costimulators ensures that T cells respond best only when necessary — that is, when faced with pathogens.

The interaction of B7-1 and B7-2 with CD28, in concert with T-cell–receptor signaling, promotes the expansion of antigen-stimulated T cells and their differentiation into effector and memory cells. CD28 is the major costimulatory receptor for naive T cells and is therefore important for initiating T-cell responses. CD28 signals enhance the pro-



Roles of the B7-CD28/CTLA-4 Pathway in Regulating T-Cell Activation.

Resting antigen-presenting cells (APCs) express few or no B7 costimulatory molecules and fail to activate naive T cells (top panel). Microbes and cytokines produced in response to microbes activate APCs and stimulate the expression of B7 costimulators. B7-CD28 interactions stimulate the expansion and differentiation of naive T cells (middle panel). CTLA-4, the high-affinity receptor for B7-1 and B7-2, is up-regulated on activated T cells. B7-CTLA-4 interactions inhibit T-cell responses (bottom panel). MHC denotes major histocompatibility complex.

duction of interleukin-2 and other cytokines, up-regulate cell-survival genes (such as *Bcl-x_L*), promote energy metabolism (glucose uptake and rate of glycolysis), and facilitate cell-cycle progression. The effects of the binding of B7 to CD28 can be mimicked by cross-linking antibodies directed against this receptor. Most of the antibodies that have been developed against CD28 in mice and humans have no biologic effects on their own but are potent co-stimulators when given with antigen or mimics of antigen. There is one notable exception, however: an anti-CD28 antibody, called a

superagonistic antibody, activates T cells in the apparent absence of the overt engagement of the antigen receptor.² The basis for this unusual action and its physiological implications are not clearly defined, but it may involve amplification of tonic T-cell-receptor signals.

B7-CD28 signals also play a critical role in the development and survival of a class of T cells called regulatory T cells, whose function is to inhibit immune responses and maintain self-tolerance. The fundamental importance of regulatory T cells in immune regulation, together with

their therapeutic potential for suppressing pathologic immune responses, has prompted investigation of their development and function.³ The development of regulatory T cells in the thymus requires CD28-stimulated production of interleukin-2. In the periphery, CD28 signaling and interleukin-2 are needed for the survival of regulatory T cells. On the basis of these findings, antibody against T-cell receptors, anti-CD28 antibody, and interleukin-2 — alone and in combination — are being used to expand regulatory T cells for cellular therapy.

Although T-cell costimulatory pathways were initially identified as stimulators of T-cell responses, it is now clear that some costimulatory receptors can inhibit T cells. Cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4) is a CD28 homologue that also binds to B7-1 and B7-2 but is expressed in an inducible fashion after T-cell activation.¹ Unlike CD28, CTLA-4 shuts off T-cell responses by inhibiting interleukin-2 production and blocking cell-cycle progression. Thus, CTLA-4 is involved in the induction and maintenance of T-cell tolerance. The critical importance of CTLA-4 as a negative regulator of the immune response is underscored by the phenotype of CTLA-4-deficient mice, which includes massive T-cell activation and proliferation and T-cell-mediated tissue damage. CTLA-4 polymorphisms in humans have been linked to susceptibility to autoimmune diseases, including type 1 diabetes and autoimmune thyroid disease.

The pivotal role of costimulation in regulating T-cell activation versus tolerance has stimulated great interest in the manipulation of costimulatory signals to either boost immune responses (in or-

der to enhance antimicrobial and antitumor immunity) or induce tolerance (in order to treat autoimmune diseases and prevent graft rejection). The therapeutic potential of manipulation of the B7-CD28 costimulatory pathway is demonstrated by the clinical success of CTLA-4-Ig, a soluble fusion protein containing the extracellular domain of CTLA-4 linked to an IgG Fc region.⁴ CTLA-4-Ig binds to B7-1 and B7-2 and works by blocking costimulation through CD28. Studies in animal models have shown that brief treatment with CTLA-4-Ig results in long-term allograft survival and amelioration of autoimmune diseases. CTLA-4-Ig (abatacept, or Orencia) was recently approved for the treatment of rheumatoid arthritis, and clinical trials for other indications are in progress. A blocking anti-CTLA-4 antibody is being tested in clinical trials as a means of stimulating antitumor immunity by reducing inhibitory signals (the converse of CTLA-4-Ig, which blocks activating signals through the B7-CD28 pathway).

The therapeutic targeting of CD28 faces fundamental challenges because of the multiple, and opposing, biologic roles of this costimulatory receptor.⁴ For instance, in most situations, the blockage or elimination of B7-CD28 signaling inhibits T-cell activation, but in at least one mouse model of type 1 diabetes, the same manipulation exacerbates the disease by eliminating regulatory T cells. Conversely, stimulation through CD28 may activate

pathogenic effector T cells as well as protective regulatory T cells. The superagonistic anti-CD28 antibody has been shown to potently activate regulatory T cells in mice and to prevent and even ameliorate disease in several mouse models.⁵

It is unclear why the superagonistic anti-CD28 antibody apparently activates pathogenic effector T cells in humans (as was revealed by the unfortunate adverse effects in the clinical trial sponsored by TeGenero) but not in mouse models (as suggested by the preclinical studies). Most pre-clinical studies are conducted in inbred mice, and their differences from outbred humans are important to consider. One difference not often appreciated is that in laboratory mice, which are typically housed in pathogen-free conditions, the majority of the T cells are naive. In striking contrast, as humans age in their microbe-rich natural habitat, the numbers of previously activated memory T cells increase. Since the activation requirements of memory cells are generally much less stringent than those of naive cells, agonistic antibodies that do not induce widespread immune activation in laboratory mice (with predominantly naive T cells) may nevertheless be capable of doing so in humans (who have more memory cells).

Another factor may be the different affinities of the anti-CD28 antibodies used in the preclinical studies and those used in the clinical trial sponsored by TeGenero. For example, the anti-human-

CD28 antibody probably binds to CD28 in nonhuman primates more weakly than it does to CD28 in humans, so the resultant T-cell stimulation may be less robust in primate T cells than in human T cells. Such considerations of affinity may be especially critical with regard to agonistic antibodies.

The use of agents that block or activate costimulatory pathways will undoubtedly require a better understanding of how the diverse biologic activities of these pathways are orchestrated, better biomarkers to predict outcomes of the manipulation of T-cell costimulation in humans, and pre-clinical studies with established predictive value. The encouraging success of CTLA-4-Ig supports the therapeutic potential of manipulating costimulatory pathways.

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