The Yin and Yang of T Cell Costimulation

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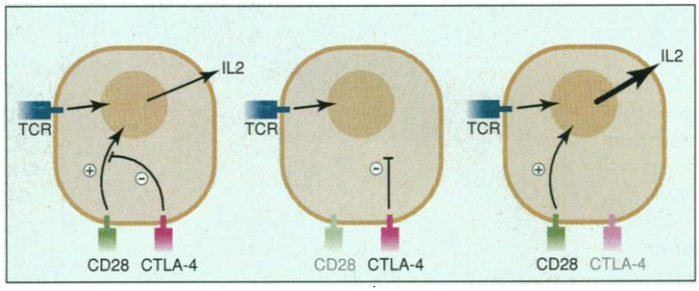
T cells require two types of signals from antigen-presenting cells (APCs) for activation and subsequent differentiation to effector function. One is an antigen-specific signal provided by interactions between the T cell antigen receptor and antigenic peptide presented by major histocompatibility complex (MHC) molecules on the APC. The second signal is an antigen-independent signal mediated by engagement of the T cell surface molecule CD28 with members of the B7 family (B7-1, CD80; B7-2, CD86) on the APC (*1*, *2*). In contrast, the purpose of another molecule on the T cell surface, the enigmatic CD28 homolog cytotoxic T lymphocyte antigen 4 (CTLA-4), has been elusive and controversial. As described on page 985 of this issue (*3*), a rampant lymphoproliferative disorder occurs in CTLA-4–deficient mice, providing compelling evidence that the molecule is critical for the negative regulation of T cell responses. This emerging story suggests that the outcome of T cell activation is determined by a delicately balanced interplay between positive signals from CD28 and negative signals from CTLA-4.

CTLA-4 was originally identified in a screen for genes involved in T cell–mediated cytolysis (*4*), but is also expressed by activated helper T cells as well. CD28 and CTLA-4 are both members of the immunoglobulin superfamily and have a nucleotide sequence homology of about 75% (*5*). The genes have similar genomic organization and are chromosomally linked in both human and mouse (*5*). Interest in the function of CTLA-4 was sparked by the demonstration that a soluble recombinant form of the protein bound B7 family members with a much higher affinity than did CD28 (*6*). With the use of monoclonal antibodies, it has become apparent that, although CD28 is constitutively expressed at high levels on all T cells, CTLA-4 expression is below the threshold of detection by flow cytometry until it is transiently detectable 2 to 3 days after activation (*7–9*).

So why do T cells have two receptors for the same ligands—the constitutive, high-abundance, low-affinity receptor CD28 and the inducible, low-abundance, high-affinity receptor CTLA-4? The data published to date have generated some controversy (*10*, *11*). CTLA-4 cannot replace CD28 in providing costimulation in CD28 mutant mice, ruling out functional redundancy (*12*). The observation that antibodies to CTLA-4 can enhance T cell proliferation raised the possibility that CTLA-4 might synergize with CD28 and sustain costimulation (*7–9*, *13*). However, antibodies to CTLA-4, when cross-linked, greatly inhibited proliferation of naïve T cells (*8*, *9*). Together, these data suggested that blockade of CTLA-4 removed, whereas aggregation of CTLA-4 provided, inhibitory signals that down-regulated T cell responses.



CD28 and CTLA-4 in T cell activation. In normal mice, the outcome of T cell activation is determined by signals from the antigen receptor, costimulatory signals from CD28, and inhibitory signals from CTLA-4. In CD28 knockout mice, the costimulatory signals are absent and T cell responses are consequently diminished. In CTLA-4 knockout mice, the CD28 costimulatory signal is unchecked, resulting in enhanced lymphokine production and proliferation.

The phenotype of the CTLA-4 knockout mice clearly supports an inhibitory signaling role for the molecule. The mice exhibit lymphadenopathy of extreme magnitude. The peripheral lymphoid organs are packed with 5 to 10 times the normal number of lymphocytes, the vast majority of which are T cell blasts expressing activation markers indicative of an ongoing immune response. In vitro, the T cells proliferate spontaneously and are hyper-responsive to T cell antigen receptor engagement. Serum immunoglobulin concentrations in the mice are orders of magnitude higher than normal, and B cells display activation markers. Extensive lymphocytic infiltration of the bone marrow, lung, liver, pancreas, and heart is evident, and the mice die by 3 to 4 weeks of age. It is not yet known with certainty whether the T cell expansion is a result of a failure to check expansion of a relatively small number of T cells responding to environmental antigens or a more generalized expansion of many T cells as a result of a lowering of the threshold for activation by self antigens. However, the fact that activated cells are equally distributed in the T cell subsets and that the T cell receptor repertoire is unaltered argue against clonal expansion of a small number of cells.

Apoptosis is a key process in homeostatic regulation of T cell responses and is triggered by activation of Fas and other members of the tumor necrosis factor receptor family (*14*). Lymphadenopathy and splenomegaly develop as a result of the accumulation of T cells in mice with mutations in Fas (*lpr*) or Fas ligand (*gld*) (*15*). The severity of the phenotype varies with the genetic background but is much less dramatic than that in the CTLA-4 knockout mice. Death usually occurs as a result of nephritis and arthritis, but only in strains with a predisposition to autoimmune disease. In contrast, the CTLA-4 knockout mice die at a young age from myocardial failure resulting from lesions caused by the infiltrating T cells. The Fas pathway is intact in the CTLA-4 knockout mice, because T cells die on exposure to antibodies to Fas, and CTLA-4 likely acts by inhibiting or terminating activation rather than by promoting cell death. Thus, Fas and CTLA-4 provide two distinct mechanisms to avoid being swamped with T cells.

The interplay between CD28 and CTLA-4 and their ligands is complex. Because CTLA-4 is expressed maximally on T cells only transiently for 2 days or so after activation, inhibitory signals generated by its engagement with B7 molecules on the APC likely terminate lymphokine production and proliferation of activated T cells (*2*, *10*). This down-regulation may rescue some T cells from activation-induced cell death, facilitating the generation of memory cells ready for a quick response when they once again encounter antigen after the decay of CTLA-4 expression. In the knockout mice, the absence of CTLA-4 would prevent termination of ongoing T cell responses. However, another (nonexclusive) possibility is that CTLA-4 can also regulate the initiation of T cell responses. Low amounts of B7 are insufficient to support T cell activation, more as a result of inhibitory signals mediated by CTLA-4 than a lack of costimulation by CD28 (*9*).

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CTLA-4 may be expressed at functionally significant levels even by unactivated T cells or be very quickly induced by antigen receptor engagement. Even at low levels of expression, CTLA-4, by virtue of its high affinity for B7, might be dominant over CD28 when limited amounts of B7 are available. This would raise the threshold for activation by self antigens. The absence of the damping effects of CTLA-4 in the knockout mice could account for the observed generalized proliferation. It seems likely that there is a dynamic interplay between antigen receptor signals and the modulating effects of CD28 and CTLA-4 throughout the early and late stages of T cell activation.

The existence of alternative pathways that regulate T cell activation offers exciting clinical prospects. Inhibition of CD28 costimulation with a soluble form of CTLA-4 can suppress immune responses, prolong organ graft survival, and ameliorate experimental autoimmune disease (*16*). Tumor cells transfected with B7 are often rejected and can induce immunity to the parental tumor cells, demonstrating that provision of costimulation can enhance host immunity to tumors (*17*). Antibody to CTLA-4 greatly enhances the clonal expansion of antigen-specific T cells in vivo (*13*). Thus, blockade of the inhibitory effects of CTLA-4 might also prove useful in augmenting T cell responses in clinical situations.

References and Notes

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Trimeric G Proteins: Surprise Witness Tells a Tale

Henry R. Bourne

Like key witnesses in a detective story, protein crystal structures can surprise, intrigue, mislead, or instruct us—and often do all four at once. On page 954 of this issue, Mixon *et al.* (*1*) serve up an intriguing surprise: Crystals of the guanosine diphosphate (GDP) form of α_{i1}, a G protein α subunit that mediates hormonal inhibition of adenylyl cyclase, reveal Gα monomers linked, head-to-tail, in endless polymers. I suspect that the inference that similar Gα polymers exist and function in vivo is a red herring. Instead, at least to this armchair sleuth, this polymer and other clues hint at a solution to a fascinating molecular puzzle at the heart of G protein signaling: How do receptors, in combination with Gβγ, trigger GDP release and G protein activation?

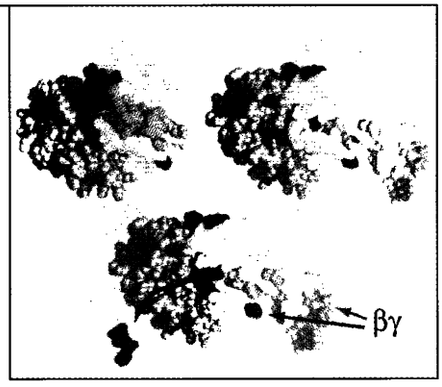
The 16 trimeric (αβγ) G proteins of vertebrates relay messages from hormone and sensory receptors to effector targets that mediate or regulate heart rate, contraction of smooth muscle, synaptic transmission, endocrine secretion, olfaction, vision, and many other functions. In each case the receptor turns on the G protein by promoting exchange of guanosine 5′-triphosphate (GTP) for GDP bound to the Gα subunit, followed by dissociation of αGTP and the Gβγ dimer from the receptor and from each other. The appropriate effector protein is then regulated by αGTP or Gβγ (or both) until bound GTP is converted to GDP, allowing αGDP to reassociate with Gβγ and turn off the signal (*2*, *3*).

Previous Gα crystal structures provided three-dimensional (3D) views of GTP-induced conformational change (*4*, *5*) and the guanosine triphosphatase (GTPase) catalytic mechanism (*6*, *7*) but no evidence for any kind of polymer. The α_{i1}GDP polymer is made possible by a small folded domain, composed of sequence from both the amino (orange) and carboxyl (yellow) termini of each monomer (the N-C domain) (see figure). Previous Gα structures showed only the Ras-like (white) and α-helical (gray) domains. In the α_{i1}GDP polymer, the new domain tucks neatly into a crevice in the α-helical domain of the next monomer. Neither the polymer nor the N-C domain was seen in α_{i1} bound to GTPγS (see figure, upper left) (*6*), suggesting that occupation of the nucleotide binding site by the GTP analog somehow promotes disorder of the amino and carboxyl termini, whereas the polymer promotes stable folding of the same sequences in the GDP-bound form. No N-C domain was seen in the GDP- or GTPγS-bound structures of α_t (*4*, *5*), a retinal Gα, presumably because the α_t amino termini were proteolytically removed before crystallization.

Is the head-to-tail polymer biologically relevant or an artefact of crystallization? I suspect the latter, although Mixon *et al.* (*1*) cite reports suggesting that G protein polymers can be detected biochemically under certain conditions in vitro. Indeed, polymeric Gα might serve as a storage form for the protein or somehow facilitate activation of many G protein molecules by a single receptor. A reason for doubt, however, is that each monomer in the polymer is rotated 180° with respect to the one preceding (*1*), allowing only alternate monomers to attach to the plasma membrane.

More disturbing is the fact that the N-C domain seen in the α_{i1}GDP polymer, if it existed in vivo, would probably prevent crucial interactions of Gα with pertussis toxin, which catalyzes adenosine 5′-diphosphate ribosylation at Cys^{351} (*2*), receptors (*8*, *9*), and Gβγ, which binds to the Gα amino terminus. Each of these would encounter steric obstacles in the polymer.

Even misleading testimony can furnish valuable clues. This is perhaps the case with the α_{i1}GDP polymer, which may not exist in cells but nonetheless reveals structural changes in the monomer (see figure, upper right) that suggest a way to resolve a para-



Three faces of $G\alpha_{i1}$. $G\alpha_{i1}$ bound to GTPγS (upper left); $G\alpha_{i1}$ bound to GDP (as in the polymer); imaginary $G\alpha_{i1}$ structure from which GDP can exit quickly.

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