Putting the brakes on BTLA in T cell–mediated cancer immunotherapy

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Commentary

Attenuating coinhibitory molecules for the treatment of cancer is gaining a great deal of attention as a strategy for immunotherapy. The B and T lymphocyte attenuator (BTLA, CD272) is a novel coinhibitory molecule structurally and functionally related to CTLA-4 and PD-1. A study in this issue of the *JCI* by Derré et al. reveals that BTLA is expressed on virus-specific human CD8\(^+\) T cells but is progressively downregulated after their differentiation from a naive to effector phenotype (see the related article beginning on page 157). Surprisingly, tumor-specific human CD8\(^+\) T cells continue to express BTLA even after their differentiation to an effector phenotype. Remarkably, vaccination of melanoma patients with CpG led to BTLA downregulation on tumor-specific human CD8\(^+\) T cells, concomitant with restoration of their functionality. We discuss these findings in the context of the expanding field of cosignaling molecules and their implications for T cell–based therapies for cancer.
Putting the brakes on BTLA in T cell–mediated cancer immunotherapy

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Attenuating coinhibitory molecules for the treatment of cancer is gaining a great deal of attention as a strategy for immunotherapy. The B and T lymphocyte attenuator (BTLA, CD272) is a novel coinhibitory molecule structurally and functionally related to CTLA-4 and PD-1. A study in this issue of the JCI by Derré et al. reveals that BTLA is expressed on virus-specific human CD8+ T cells but is progressively downregulated after their differentiation from a naive to effector phenotype (see the related article beginning on page 157). Surprisingly, tumor-specific human CD8+ T cells continue to express BTLA even after their differentiation to an effector phenotype. Remarkably, vaccination of melanoma patients with CpG led to BTLA downregulation on tumor-specific human CD8+ T cells, concomitant with restoration of their functionality. We discuss these findings in the context of the expanding field of cosignaling molecules and their implications for T cell–based therapies for cancer.

Two families of cosignaling molecules, the CD28 family and the TNF receptor (TNFR) family, are master regulators of the immune system at the cell surface (1–3). During cell-to-cell contact, specific recognition occurs between various cosignaling molecules, and this interaction ignites a plethora of signaling events, resulting in either the activation (costimulation) or the attenuation (coinhibition) of T cell function and proliferation (Figure 1). Indeed, cosignaling molecules are among the first responders of the immune system to self, foreign, and tumor antigens. A key feature of cosignaling molecules is that their functions are dependent on the TCR signal, and these cosignaling molecules (often referred to as “signal 2”) are necessary to direct, modulate, and fine-tune the TCR signal (often referred to as “signal 1”).

Overview of lymphocyte cosignaling

It has been nearly 40 years since Bretscher and Cohn first proposed the “two-signal” model for T cell activation, and while the details have become increasingly complex, the simplicity of the model provides a basic framework with which to understand mechanisms that maintain immune tolerance (4). In the mid-1980s, CD28 was identified as the first cosignaling molecule. CD28 provides the dominant signals required for full activation of naive lymphocytes and thus is called a costimulatory molecule. Shortly after the discovery of CD28, cytotoxic T lymphocyte antigen–4 (CTLA-4) was identified as a protein that shares ligands and structural homology with CD28 (5). In contrast to CD28, however, CTLA-4 was found to inhibit T cell responsiveness and is thus considered a coinhibitory molecule. Manipulation of the CTLA-4 pathway using antibody blockade has shown considerable promise for the treatment of patients with cancer, and these clinical data have motivated investigators to search for other coinhibitory molecules for clinical benefit.

Several additional coinhibitory molecules have been identified in the past decade, including programmed death–1 (PD-1), lymphocyte activation-gene 3 (LAG-3), CD160, and the B and T lymphocyte attenuator (BTLA). BTLA is the most recently identified receptor of the CD28 family and is structurally related to CTLA-4 and PD-1 (6). BTLA binds the herpes virus entry mediator (HVEM). Interestingly, HVEM is a member of the TNFR family; and its interaction with BTLA is the first demonstration of crosstalk between the CD28 and TNFR families (7), an observation that has profound implications for the complexity of regulation of the innate and acquired immune systems.

In contrast to mice lacking CTLA-4 or PD-1, young BTLA-deficient mice show no obvious signs of autoimmunity; however, with age, they develop autoimmune hepatitis and other signs of immunopathology (8). Furthermore, mice lacking BTLA are far more susceptible to EAE, a model of T cell–mediated autoimmune disease that shares several features with MS (6). Interestingly, BTLA blockade prevents proliferation and cytokine production by T cells, while BTLA triggering leads to decreased antimicrobial and autoimmune responses in mice, suggesting that BTLA may have an important role in restraining cellular immunity. However in humans, little is known about the contribution of BTLA to tolerance and immunopathology, or to its functional effect on antigen-specific T cells in vivo.

Cancer patients have abnormal expression of BTLA

In this issue of the JCI, Derré and coworkers report that naive human CD8+ T cells express high levels of BTLA on their cell surface (9). However in bulk and influenza-specific CD8+ T cells in healthy donors and cancer patients, they found that the surface expression of BTLA is gradually downregulated during differentiation of human CD8+ T cells from the naive to effector cell phenotype (Figure 2A). Remarkably, this is not the case for tumor-specific human CD8+ T cells. In sharp contrast, these cells persistently expressed high levels of BTLA in vivo and remained susceptible to functional inhibition by the BTLA ligand HVEM (Figure 2B). Importantly, the authors demonstrate abundant expression of HVEM in a subset of melanomas and show that tumor-specific CD8+ T cells in these patients were inhibited by interaction of BTLA with HVEM on the tumor, even after progressive differentiation of the T cells.

Additional investigation by Derré et al. revealed persistent BTLA expression on spontaneously elicited tumor antigen Melan-A/MART-1–specific CD8+ T cells from melanoma patients and after conven-
tional peptide vaccination consisting of a Melan-A26-35 peptide and incomplete Freund’s adjuvant (9). As modeled in Figure 2B, these cells possess minimal functionality upon tumor encounter. Surprisingly, the addition of a TLR9 agonist, CpG, to the vaccine comprising Melan-A26-35 peptide and incomplete Freund’s adjuvant led to “normalization”: progressive BTLP downregulation in vivo on those cells and resistance to BTLP-HVEM–mediated inhibition, as assessed by the capacity of these cells to produce IFN-γ after recognition of tumor antigen (Figure 2C).

This work (9) catapults the field of tumor immunotherapy forward by demonstrating, for the first time to our knowledge, that BTLP is a valid target for cancer immunotherapy. The coinhibitory molecule BTLP can inhibit tumor-specific human CD8+ T cells; and vaccination with CpG adjuvants, at least in part, overcomes this barrier by downregulating BTLP. CpG-mediated downregulation of BTLP correlates with restoration of the in vivo effector function of tumor-specific human CD8+ T cells. These data underscore the therapeutic potential of exploiting the BTLP pathway to treat patients with cancer and infectious disease as well as patients with autoimmunity. Thus, a therapeutic immune intervention to treat infectious or malignant disease would involve blocking BTLP-mediated T cell inhibition, whereas this pathway could be pharmacologically augmented to promote BTLP-mediated T cell inhibition or tolerance for patients with autoimmune diseases or for allograft recipients.

**Exciting findings lead to new questions**

The current studies raise fundamental questions for further investigation. The present
BTLA expression inhibits tumor-specific human CD8\(^+\) T cell function, which can be overcome by vaccination with CpG. In this issue of the *JCI*, Derré et al. report that naive human CD8\(^+\) T cells express high levels of the coinhibitory molecule BTLA on their surface. They find that BTLA can inhibit the function of tumor-specific human CD8\(^+\) T cells. In vivo, vaccination of melanoma patients with CpG dampened this inhibition, at least in part, by downregulating BTLA. (A) The surface expression of BTLA is gradually downregulated during differentiation of virus-specific human CD8\(^+\) T cells from a naive (CCR7\(^{+}\)CD45RA\(^{+}\)) to an effector cell phenotype (CCR7\(^{-}\)CD45RA\(^{-}\)). These differentiated cells produce high amounts of IFN-\(\gamma\). (B) The surface expression of BTLA is maintained on tumor Melan-A\(^{MART-1}\)-specific human CD8\(^+\) T cells from patients vaccinated with conventional peptide vaccination consisting of a Melan-A\(^{26-35}\) peptide and incomplete Freund’s adjuvant (IFA) even after their differentiation to an effector phenotype, and this is associated with impaired functionality, as indicated by their reduced capacity to produce IFN-\(\gamma\). (C) The vaccination of melanoma patients with the TLR agonist CpG led to progressive BTLA downregulation on tumor Melan-A\(^{MART-1}\)-specific human CD8\(^+\) T cells and resistance to BTLA-HVEM-mediated functional inhibition and robust production of IFN-\(\gamma\). These data not only underscore the therapeutic potential of CpG but also reveal the clinical importance of the BTLA pathway.
Figure 3

Models of interaction among HVEM, BTLA, CD160, and LIGHT and their various functional effects on tumor-specific human CD8+ T cells. BTLA, CD160, and LIGHT are differentially expressed on tumor-specific CD8+ T cells, and depending on their expression, they can mediate distinct outcomes: immune tolerance or effective immunity against tumor targets. Three potential interactions are shown. Left: If BTLA or CD160 is expressed and LIGHT expression is either low or absent, the coinhibitory BTLA-CD160-HVEM complex will be dominant, resulting in negative regulation of the tumor-specific CD8+ T cell by the human tumor. Middle: If LIGHT, BTLA, and CD160 are all expressed, they might form a complex with HVEM. This could trimerize HVEM, resulting in positive or negative regulation of the T cell by the tumor. Right: If LIGHT is expressed with little to no BTLA or CD160, the tumor-specific T cells receive a positive signal from the HVEM-expressing tumor, resulting in robust functional activation of the tumor-specific T cell. Thus, attenuation of BTLA via either CpG or antibody blockade might augment T cell–mediated immunotherapies of cancer. Potent tumor-specific T cell responses are mediated with conventional vaccination and CpG, which downregulates BTLA expression on T cells, as revealed by new findings reported by Derré et al. (9) and as represented in the right panel. Adapted with permission from Trends in Immunology (22).

Sodium channels gone wild: resurgent current from neuronal and muscle channelopathies

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Voltage-dependent sodium channels are the central players in the excitability of neurons, cardiac muscle, and skeletal muscle. Hundreds of mutations in sodium channels have been associated with human disease, particularly genetic forms of epilepsy, arrhythmias, myotonia, and periodic paralysis. In this issue of the JCI, Jarecki and colleagues present evidence suggesting that many such mutations alter the gating of sodium channels to produce resurgent sodium current, an unusual form of gating in which sodium channels reopen following an action potential, thus promoting the firing of another action potential (see the related article beginning on page 369). The results of this study suggest a widespread pathophysiological role for this mechanism, previously described to occur normally only in a few types of neurons.

Our understanding of channelopathies—human disorders arising from mutations of ion channel genes—has gone through several waves of discovery. First, there was the implication that ion channels may play a causal role in disease pathology from the observation of abnormal ionic conductances in muscle biopsied from individuals with myotonia or periodic paralysis, studied using microelectrode recording (1, 2). Then came identification of mutations in ion channel genes, made possible by discovery of ion channel gene superfamilies; disease-associated mutations were identified by genome-wide linkage studies or by a candidate gene approach guided by the discovery of aberrant conductances in affected cells (3). This approach enabled the identification of numerous channelopathies in heart,