

Costimulation of effector CD8⁺ T cells: which receptor is optimal for immunotherapy?

Abstract

To induce strong immune responses, naive CD8⁺ T cells require stimulation through the TCR and costimulatory receptors. However, the biological consequence of activating costimulatory receptors on effector T cells is still unclear. In addition, activating CD8⁺ T cells either with vaccination or adoptive transfer of activated or gene-modified T cells are novel approaches for cancer and antiviral therapies. To enhance T cell efficacy, activation of costimulatory receptors is often incorporated in therapeutic designs; however it is still unclear how stimulation of different costimulatory receptors influences T cell function. Therefore it is essential to study how different costimulation receptors alter the gene expression and functions of activated CD8⁺ T cells. This will determine how combinations of costimulatory signals shape immunity and how to best activate and utilize these receptors for immunotherapy.

Keywords: immunotherapy, costimulation, CD8 T cell; CD28, NKG2D

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Abbreviations: TCR, T cell receptor; APC, antigen presenting cell; NKG2D, natural killer group 2 D; NK cell, natural killer cell; PI3K, phosphatidylinositol,3 kinase; IFN, interferon; IL, Interleukin; TNF, tumor necrosis factor; NF- κ B, nuclear factor kappa B; mTORC1, mammalian target of rapamycin 1 complex; NFAT, nuclear factor of activated T cells; GSK-3 β , glycogen synthase kinase-3 beta; TRAF, TNF receptor-associated factor; MAPK, mitogen-activated protein kinase; CAR, chimeric antigen receptor

Short communication

While the most recognized function of CD8⁺ T cells is their ability to directly kill infected or tumor cells, activated CD8⁺ T cells are also a significant source of immune-modulating cytokines, making these cells essential players in immune responses to viruses and tumors. It is well known that upon the first encounter with antigen, complete activation of naive CD8⁺ T cells requires stimulation through the T cell receptor (TCR) and costimulatory receptors. Costimulatory receptors expressed on T cells bind to ligands on antigen presenting cells (APCs) or target cells, and these ligands are often expressed upon infection, cell stress, or transformation. The nature of costimulation and other signals during APC-T cell interaction shapes the generation of the effector T cell response. Typically, the strongest activation of naive CD8⁺ T cells results from stimulation through the CD28 receptor which promotes T cell proliferation and survival through secretion of IL-2 and expression of anti-apoptotic proteins including Bcl-xL.^{1,2} However there are many other activating costimulatory receptors expressed on CD8⁺ T cells that also play a critical role in shaping T cell function, including the NKG2D, OX-40, and 4-1BB receptors.^{3,4} Once a T cell has been activated, it is recruited to the site of infection or tumor where it performs effector functions to destroy the target cells. Stimulation through the TCR is still required for activation of effector CD8⁺ T cells. In addition, continued activation of costimulatory receptors at the target site also likely alters the CD8⁺ T cell effector response and may determine the outcome of the immune response to tumors and infections. However the biological consequence of stimulating these receptors on activated CD8⁺ T cells is still not well understood. Therefore a major area of current research focuses on elucidating how stimulation of costimulatory receptors alters the functions of effector CD8⁺ T cells.

The NKG2D costimulatory receptor is currently a focus of anti-tumor, anti-viral, and auto-immunity studies. NKG2D is an activating receptor expressed on NK cells, all human CD8⁺ T cells, activated murine CD8⁺ T cells, $\gamma\delta$ T cells, and some CD4⁺ T cells.^{5,6} In T cells, NKG2D associates with an adaptor protein, DAP10, and provides a costimulation signal by activating intracellular signaling pathways.⁷⁻⁹ The cytoplasmic domain of DAP10 has one known signaling motif, a YINM-sequence, that is also found in the CD28 costimulatory receptor.^{8,9} After receptor stimulation, the tyrosine is phosphorylated and phosphatidylinositol-3 kinase (PI3K) and a Grb2-Vav1 complex are subsequently activated, leading to downstream Akt activation and mitogen-activated protein kinases (MAPKs) respectively.⁹ Akt activation by either of these two receptors initiates a plethora of downstream signaling pathways including NF- κ B, mTORc1, NFAT, GSK-3 β , and many others depending on what other proteins are being activated concurrently. NKG2D/DAP10 and CD28 differ in that DAP10 lacks additional signaling domains responsible for the binding of other signaling molecules including Itk, Tec, and Lck.¹⁰ Previous studies have shown that NKG2D induces similar but not identical effects to CD28 in naive and effector CD8⁺ T cells, further suggesting that the activation of signaling and resulting gene expression may not be identical between the two receptors.^{7,8,10,11} Specifically, recent work completed has shown that compared to CD28 stimulation, NKG2D stimulation in human and murine effector CD8⁺ T cells decreased the expression and secretion of anti-inflammatory cytokines IL-10, IL-9, IL-13 through activation of the β -catenin pathway.¹¹ Additionally, stimulation of NKG2D on CD8⁺ T cells by NKG2D ligands expressed on tumor or virally-infected cells leads to an increased secretion of proinflammatory cytokines including IFN- γ and TNF- α , increased killing of target cells by CD8⁺ T cells, and development of CD4-independent CD8⁺ T cell memory responses.¹⁰⁻¹⁶ It is likely that the differential activation of signal transduction pathways by CD28 and NKG2D receptors changes the gene expression profiles and functions of effector T cells, but the mechanisms of how this occurs are still unknown.

While CD28 and NKG2D receptors are often the subject of costimulation studies, they are not the only costimulatory receptors expressed on activated CD8⁺ T cells. Two other activating receptors expressed by effector T cells are 4-1BB and OX-40, both of which are

members of the tumor necrosis factor receptor family.^{2,17-19} Similar to CD28 and NKG2D, both of these receptors provide signals to enhance T cell responses and they play a key role in T cell survival, cytokine secretion, and the development of CD8 T cell memory responses. However, the initial activation of signal transduction pathways by these two receptors is quite different from CD28 and NKG2D receptors. 4-1BB recruits TRAF1 and TRAF2 adaptors, and 4-1BB signals are mediated mainly by the activation of NF- κ B, c-Jun and p38 downstream pathways.¹⁸ OX40 binds to and activates TRAF2, 3, and 5 as well as PI3K/Akt and NF- κ B.¹⁹ In comparison, the intracellular signaling of CD28 and NKG2D pathway occurs mainly through activation of PI3K/Akt, NF- κ B, and the MAPKs, but are not known to activate TRAF proteins.¹⁹ Each of these receptors ultimately promotes effector T cell proliferation, survival, and cytokine production, as well as the generation and maintenance of memory T cells. However, each receptor seems to induce these functions to a different degree causing a unique activation status.^{3,4,11} It is likely that the differential signaling of these receptors changes the gene-expression profiles and effector functions in T cells, but the details of these mechanisms are still not clear.

Elucidating the effect of differential costimulation in activated CD8⁺ T cells is important for many reasons. A majority of the work conducted on the activation cascades initiated by costimulatory receptors has been performed in naive CD8⁺ T cells. However, at an infection or tumor site activated T cells will likely be stimulated through these receptors and the resulting changes in gene expression will alter their effector functions. CD28, NKG2D, OX-40, and 4-1BB are all expressed on activated CD8⁺ T cells.^{3,4} The ligands for these receptors are also likely expressed on cells present at the effector site. The expression of ligands for CD28 and 4-1BB is restricted to activated professional APCs, such as dendritic cells, macrophages, and B cells, while the ligand for OX-40 can be induced on professional APCs as well as on T cells, Langerhans cells, mast cells, NK cells, endothelial cells, and smooth muscle cells.^{3,4} Many of these cells that express the ligands are present at the infection or tumor site, thus these receptors are likely stimulated during effector responses. Comparatively, the NKG2D receptor binds to ligands that are induced by DNA damage and cell stress. These ligands can be expressed by multiple types of cells and are present on many types of tumor cells, in autoimmune diseases, and during some infections.^{5,20} Compared to the other three costimulatory receptors whose ligand expression is restricted to a few cell types, it is highly likely that the NKG2D receptor is stimulated on activated CD8⁺ T cells during an immune response. Overall, all of these receptors, particularly NKG2D, are likely activated on effector CD8⁺ T cells at an infection or tumor site. Therefore it is essential to learn how these receptors alter gene expression and function of activated T cells and ultimately shape the immune response.

In addition to learning about the basic biology controlling T cell activation, this topic is of great importance because these costimulatory receptors are targets for many therapies. One current focus is to use costimulation to enhance T cell function for cancer therapy with multiple targeting approaches being tested.^{3,4,17} One approach uses agonistic antibodies or ligand-Fc fusion proteins to stimulate costimulatory receptors on T cells. In particular stimulating OX-40 or 4-1BB with either of these soluble protein-based modalities has shown significant activation of CD8⁺ T cells and reduction of tumors in clinical trials, although some studies suggest that OX-40 targeting may have stronger effects on CD4⁺ T cells.²¹⁻²³ NKG2D ligand-Fc fusion proteins have also shown significant anti-tumor efficacy in mouse models.²⁴ Therefore, direct *in vivo* activation of costimulatory

receptors on CD8⁺ T cells seems to enhance tumor reduction likely through altering effector CD8⁺ T cell functions.

A second mechanism used to activate costimulatory receptors during cancer therapy is to include the ligands for costimulatory ligands cancer vaccines.^{3,4,21} Several phase I and phase II clinical trials evaluated the efficacy of tumor cell vaccines that were transfected with B7-1, one of the ligands for CD28, and showed some evidence of anti-tumor efficacy in metastatic renal carcinoma and non-small cell lung cancer.^{3,25,26} B7-1 has also been included in tumor antigen vaccines targeting antigens such as carcinoembryonic antigen (CEA), mucin-cell-surface associated 1 (MUC-1), and prostate-specific antigen (PSA) antigens, with promising results in initial preclinical studies, meaningful clinical improvement has been limited, likely due to concurrent inhibition of T cells through the CTLA-4 inhibitory receptor.^{3,27-29}

A third approach is to incorporate costimulatory domains in chimeric antigen receptors (CARs) (30-32). These receptors, either Fc- or receptor- based, are used to redirect T cell specificity and activation to enhance tumor cell targeting and destruction. CD28, Dap10/NKG2D, OX-40, and 4-1BB have all been incorporated into various chimeric antigen receptors.³⁰⁻³² Second generation CARs typically consist of coupling CD3 ζ with one costimulatory domain and third generation CARs incorporate two or more domains.³⁰⁻³² In general incorporation of any costimulation domain in second generation CARs increased inflammatory cytokine secretion, including production of IFN γ , GM-CSF, and TNF α , T cell survival through expression of anti-apoptotic molecules (Bcl-xL), and tumor cell cytotoxicity compared to the first generation CARs whose signal transduction domains consisted of only CD3 ζ or other activating domains. However, when directly compared to each other, varying results for tumor killing, cytokine secretion, trafficking, and persistence were seen depending on which costimulatory domain was incorporated and which tumor type was being tested.

While the optimum combination of signaling domains is still not known, what is clear from these studies is that no one costimulatory domain is superior. Instead it seems that the differences in T cell function induced by these receptors work in a tumor-specific manner, with one combination of signals not being ideal for every tumor type.³²⁻³⁵ Thus, the best combination of anti-tumor functions will likely have to be optimized for each tumor. However, before we can select which costimulatory receptor is ideal for anti-cancer therapies, we first must discover the differences in gene expression and resulting functions induced by each costimulatory receptor.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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