The role of CD27 in anti-viral T-cell immunity

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Abstract

CD27 is a co-stimulatory immune-checkpoint receptor, constitutively expressed on a broad range of T-cells (αβ and γδ), NK cells and B-cells. Ligation of CD27 with CD70 results in potent co-stimulatory effects. In mice, co-stimulation of $CD8^+$ T-cells through CD27 promotes immune activation and enhances primary, secondary, memory and recall responses towards viral infections. Limited *in vitro* human studies support mouse experiments and show that CD27 co-stimulation enhances antiviral T-cell immunity. Given the potent co-stimulatory effects of CD27, manipulating CD27 signalling is of interest for viral, autoimmune and anti-tumour immunotherapies. This review focuses on the role of CD27 co-stimulation in anti-viral T-cell immunity and discusses clinical studies utilising CD27 co-stimulation pathway for anti-viral, anti-tumour and autoimmune immunotherapy.

Keywords:

CD27, CD70, anti-viral immunity, co-stimulation, T-cells, immunotherapy

Introduction

CD27 is a receptor of the tumor necrosis factor (TNF) superfamily, expressed on a broad range of lymphocytes, including T-cells (αβ and γδ) [1-3], B-cells [4-6] and natural killer (NK)-cells [7,8]. In T-cells, binding of CD27 to its ligand CD70 results in activation of both canonical and alternative NFκB pathways [9,10] that mediate signalling and downstream co-stimulatory effects and provide potent enhancement of T-cell responses [11,12]. CD27 co-stimulation promotes immune responses and enhances primary, secondary, memory and recall $CD8^+$ T-cell responses towards acute viral infections in murine models [10,13]. However, the role of CD27 in human lymphocytes is understudied. Due to its strong co-stimulatory effects, the CD27/CD70 pathway has recently gained interest as an immunotherapeutic target for anti-viral immunity. Manipulation of this pathway may also be beneficial for the control of autoimmune diseases or tumour immunotherapy. This review summarizes the impact of CD27 co-stimulation in anti-viral T-cell immunity and discusses its potential for immunotherapies.

CD27 expression and its potent role in T-cell activation

(i) Optimal T-cell activation requires 3 signals

Following thymic selection, naive circulating T-cells survey for foreign antigens displayed by professional antigen presenting cells (APCs), mainly dendritic cells (DCs) [14]. During infection, DCs acquire antigens, either through direct infection or uptake of material from infected tissues, become activated and migrate to secondary lymphoid tissues where they present pathogen-derived peptides to circulating T-cells. DCs typically require an initial interaction with antigen-specific helper $CD4^+$ T-cells [15,16] before they are licensed to activate naïve $CD8^+$ T-cells [16,17]). Effective activation of naïve $CD8^+$ T-cells by licensed DCs requires three distinct signals [18,19]. $CD8^+$ T-cells recognize the pMHCI complex through use of their T-cell receptors (TCRs) to provide the first signal [20]. The second signal is provided by co-stimulation via the interaction of TNF-TNFR family receptors [21] and CD28-CD80/86 [22] on the $CD8^+$ T-cell and DCs [19,23]. Lastly, pro-inflammatory cytokines (mainly IL-12 and Type I IFN) present in the environment during priming provide a third signal that can influence subsequent T-cell differentiation pathways to ensure a productive response [24]. The presence of all three signals activates a

cascade of signalling pathways, culminating in the activation and translocation of NFκB to the nucleus of T-cells [25]. This induces T-cell proliferation and differentiation, resulting in the acquisition of effector functions and modification of cell surface markers, including cytokine/chemokine receptors and integrins that enable migration to the site of infection [26].

(ii) The role of CD27 co-stimulation in T-cell activation

While studies to date have focused predominantly on co-stimulation via CD28, more recently the role of CD27 co-stimulation in T-cell activation has been acknowledged [9,13]. CD27, first characterised by van Lier *et al* [27] in 1987, is a co-stimulatory molecule in both mice [28] and humans [27,29]. It is a transmembrane homodimer of the TNFR family [9,30,31], constitutively expressed on the surface of progenitor and naïve T-cells, as well as subsets of NK- and B-cells [9]. Its ligand, CD70, is inducible on APCs, DCs [32], B cells (triggered by TLR4/9, IFNγ and CD40) and T-cells (following TCR interactions in the presence of CD28 cross-linking) and is constitutively expressed on smooth muscle cells [9]. Following the interaction between CD27 and CD70, TNFR-associated factor (TRAFs) adaptors are recruited [33], which then activate $CD8^+$ T-cells through both canonical and alternative NFκB pathways [9,10]. The CD27-CD70 interaction also induces the up-regulation of anti-apoptotic molecules (BCL-XL) [34] and cytokine receptors (IL-2Rα and IL-12Rβ), thus increasing $CD8^+$ T-cell sensitivity to cytokines [9]. This interaction facilitates activation of JuN N-terminal kinase (JNK), activator protein 1 (Ap1), eRK and mitogen activated protein (MAP) kinases to promote cytokine production including: IL-2, IL-4, IL-5, IL-6, IL-12, IFNγ and TNFα [9]. CD27 expression on T-cells increases following activation and is accompanied by release of a soluble extracellular part of the molecule [35]. Loss of CD27 expression on T-cells is observed during prolonged stimulation and is associated with fully a differentiated effector phenotype.

CD27 co-stimulation enhances antiviral T-cell immunity

Analyses in mice suggest that co-stimulation through CD27 is important during T-cell development [36], primary activation [11,37*-41], transition into memory [11,37*,42], secondary recall and the long-term survival of T-cells [13,31] (summarised in Figure 1 and Table 1). Gravestein *et al* [36] observed CD27

expression on thymocytes during the double negative (DN) stage of development and using $RAG^{-/-}$ mice showed that blocking CD27 co-stimulation with a mAb decreased the transition of DN to double positive (DP) thymocytes, thus revealing that CD27 co-stimulation is important in T-cell development.

(i) CD27 co-stimulation enhances primary anti-viral $CD8^+$ T-cell responses

Published evidence reveals the importance of CD27 co-stimulation during primary viral infection (Table 1). Willoughby *et al* [37*] and Rowley *et al* [11] adoptively transferred OT-1 $CD8^+$ T-cells into naïve mice and activated them with OVA peptide in the presence of a CD27-agonist antibody. Augmented CD27 co-stimulation increased the expansion of epitope-specific $CD8^+$ T-cells ~50-fold [37*], improved effector function and enhanced cytotoxicity in response to re-stimulation with peptide [11]. Similarly, using CD70-transgenic mice with constitutive CD70 expression on T-cells [41], CD27 co-stimulation resulted in a ~2-fold increase in the number and function of $D^bNP_{366}{}^+CD8^+$ T-cells and accelerated viral clearance following influenza A virus (IAV) infection. Conversely, in $CD27^{-/-}$ mice, the number of total $D^bNP_{366}{}^+CD8^+$ T-cells was decreased in the lungs at 10 days after IAV infection [38]. Furthermore, T-cells isolated from $CD27^{-/-}$ mice were less likely to proliferate compared to T-cells isolated from wild-type (WT) mice following anti-CD3 cross-linking *in vitro*. Interestingly, anti-CD28 co-stimulation augmented this proliferation, but not to WT levels, suggesting that CD27 and CD28 co-stimulation are not redundant and are qualitatively different. $CD70^{-/-}$ mice infected with acute LCMV displayed a <2-fold decrease in total $D^bNP_{396}{}^+CD8^+$ T-cells 6-8 days post infection (dpi) and a ~5-fold reduction in viral clearance compared to WT mice [39*]. Similarly, $CD8^+$ T-cells from WT mice infected with acute LCMV and treated with a blocking CD70 mAb [40] were less functional upon peptide re-simulation at 7dpi. Collectively, these studies demonstrate that CD27 co-stimulation is important for $CD8^+$ T-cell proliferation, cytotoxicity and function and enhances viral clearance during primary infection with acute IAV and LCMV.

(ii) CD27 co-stimulation augments memory $CD8^+$ T-cell pools

CD27 co-stimulation also increases the magnitude of memory epitope-specific $CD8^+$ T-cell populations. In adoptive transfer experiments with OT-1 $CD8^+$ T-cells,

augmenting CD27 co-stimulation during priming increased the proportion of OT-1 $CD8^+$ T-cells >30-fold at 23 days post-activation (dpa) [11]. Conversely, chronic CD27 co-stimulation decreased $NP_{366}{}^+CD8^+$ T-cell numbers 4-fold 57dpi with IAV [41]. The increase in epitope-specific memory with CD27 co-stimulation is likely to result from enhanced IL-7 signalling, as augmentation of CD27 co-stimulation retains IL-7Rα expression on T cells [37*], while blocking CD27 co-stimulation decreases IL-7Rα expression [42]. IL-7, produced by non-hematopoietic cells (e.g. stromal and epithelial cells) and immune cells such as DCs (reviewed in [43]) is functionally important for memory cell development and survival [44]. In this way, CD27 co-stimulation increases sensitivity to IL-7 via IL-7Rα expression and enhances epitope-specific $CD8^+$ T-cell transition into memory.

(iii) $CD8^+$ T-cell recall is increased with CD27 co-stimulation

CD27 co-stimulation also enhances $CD8^+$ T-cell recall (Table 1). Blocking CD27 co-stimulation in $CD27^{-/-}$ mice delayed $CD8^+$ T-cell recall following secondary IAV infection, with an early reduction in virus-specific $CD8^+$ T-cells observed 5dpi [38]. However, this difference was reduced by 7dpi. Conversely, augmenting CD27 co-stimulation either during priming [11] or recall [45*] enhanced secondary responses by OT-I $CD8^+$ T cells to OVA peptide. Interestingly, constitutive CD27 co-stimulation resulted in diminished T cell responses and impaired protection following secondary challenge with IAV [41]. These data show that enhanced, but not constitutive, CD27 co-stimulation during either primary or secondary infection can augment memory formation and recall responses.

(iv) Effects of CD27 co-stimulation in humans

Despite numerous murine studies, little is known about the role of CD27 co-stimulation in human T-cells (Table 2). *In vitro* findings show that augmenting CD27 co-stimulation by CD27 cross-linking [46,47**] or the addition of Colo679-CD70-expressing cells [48] increased proliferation and function (IFNγ/TNF expression) of human T-cells 2- to 4-fold following non-specific activation [46,48]. Expectedly, gene expression profiling showed activation and proliferation profiles in T-cells with enhanced CD27 co-stimulation [47**]. Two independent studies also correlated the loss of CD27 co-stimulation with disease severity. A total of 8 patients with severe infectious mononucleosis (IM) and complications including EBV-associated

proliferative disorder and HLH malignant lymphoma, had mutations in their CD27 gene [49,50], resulting in loss of expression and thus CD27 co-stimulation. These studies suggest that CD27 co-stimulation is important in controlling chronic EBV infection and that CD27 co-stimulation has similar effects in mice and humans, and thus CD27 is important for effective activation of human $CD8^+$ T-cells.

CD27 expression on γδ T-cells, B-cells and NK-cells

(i) Expression of CD27 on murine and human γδ T-cells

Recently, it became apparent that two functionally distinct subsets of γδ T-cells display differential expression of CD27. Although both $CD27^+$ and $CD27^-$ subtypes produce IFN-γ, only $CD27^-$ γδ T-cells produce IL-17 following *in vitro* stimulation with phorbol 12-myristate 13-acetate (PMA) and ionomycin [1]. This effect of CD27 expression on γδ T-cells was characterised on thymocytes derived from foetal organ thymic cultures (FTOC) and showed that $CD27^+$ γδ thymocytes had higher *Ifng* mRNA expression, while $CD27^-$ γδ thymocytes had decreased *Il17* expression (Table 3). Furthermore, $CD27^-$ γδ T-cells isolated from the spleen, lymph nodes (LN), lung or gut were $CD44^{hi}$ and $CD62L^{lo}$, whereas $CD27^+$ γδ T-cells had lower CD44 expression, supporting CD27 as a marker of γδ T-cell differentiation with distinct functional outcomes [1]. Lombes *et al* [3*] reported that innate-like IL-17-producing $CD27^-$ γδ T-cells correspond to Ly-$6C^-CD44^{hi}$ γδ T-cells, as they proliferate in secondary lymphoid organs and thus have self-renewing, long-living properties.

The impact of the CD27 co-stimulatory pathway in peripheral γδ T-cells remains largely unknown. Lombes *et al* [3*] suggested that peripheral $CD27^+$ γδ T-cells are similar to αβ T-cells, by having distinct naïve-like and memory-like subsets with characteristic phenotypic, functional, and homeostatic outcomes, and as such, CD27 co-stimulation may affect the $CD27^+$ γδ T-cells in a similar manner. This was addressed by Ribot and colleagues [51], who stimulated murine $CD27^+$ γδ T-cells *in vitro* with sCD70 and showed increased IFNγ/TNF production with CD27 co-stimulation. Additionally, accumulation of IFNγ-producing $CD27^+$ γδ T-cells during MuHV-4 herpes or malaria infection was dependent on CD27 expression [51], emphasising the importance of CD27 for both anti-viral and anti-parasitic immunity (Table 3). Finally, to our knowledge, only one study has explored the co-stimulatory role of CD27 on human peripheral blood γδ T-cells. deBarros *et al* [52] showed that

interaction of CD27 with its ligand CD70 resulted in increased TCR-dependent activation in γ9δ2 T-cell lines, increased proliferation, enhanced survival and cytokine production, establishing the importance of CD27 co-stimulation on the functional differentiation of human γ9δ2 T-cells (Table 3). Since γδ T-cells contribute to anti-viral and anti-cancer immunity, it is important to elucidate the role of CD27 co-stimulation and its potential role for future immunotherapies.

(ii) CD27 as a memory B-cell marker

Like T-cells, human B-cells can be subdivided according to their CD27 expression. However, unlike T-cells, naïve B-cells do not express CD27 and instead expression of CD27 is associated with memory. Interestingly, $CD27^-$ B-cells populate the non-mutated V gene compartment of naïve-like B-cells [4], while expression of CD27 ($CD27^+$) is commonly used to identify human memory B-cells with mutated V genes [4,5]. Circulating $CD27^+$ memory B-cells can be further subdivided by their relative expression of the immunoglobulin (Ig) antibodies IgM and IgD [4,53]. Up to 40% of human peripheral B-cells express CD27 and show mutated variable regions in their Ig genes [6], making the CD27 receptor an interesting marker for B-cell subsetting. However, a minor $CD27^-$ memory B-cell subset makes up 1-4% of all peripheral B-cells [6]. Therefore, CD27 expression alone is insufficient for B-cell memory identification. The function of CD27 co-stimulation on human memory B-cells has not been characterised, however, it is thought that, similar to T-cells, CD27-receptor signalling in B-cells can enhance survival [12]. It would be interesting to understand whether CD27 co-stimulation on B-cells is beneficial for maintaining lifelong serological memory by promoting the survival of memory B-cells. As such, determining the functional role of CD27 co-stimulation for B-cells may provide further potential for CD27-based immunotherapies.

(iii) CD27 expression marks functionally distinct NK-cells

NK-cells can also be sub-divided based on CD27 expression in both mice and humans [7,8]. In mice, the presence or absence of CD27 expression results in distinct effector functions, proliferative capacities, responsiveness, interaction with DCs, and migratory activity of NK-cells [7]. $Mac1^{high}CD27^+$ murine NK-cells show increased IFNγ production compared to $CD27^-$ NK-cells following activation with the NKG2D

ligand or IL-12 and IL-18. $CD27^+$ NK-cells are predominately located in lymphoid organs and are considered to be naïve, while $CD27^-$ NK-cells are located in the lung or peripheral blood and represent long-lived or senescent NK-cells [7].

Accordingly, two subsets of NK-cells, based on CD27 expression, are found in humans [8]. The majority of circulating human peripheral blood NK-cells are $CD27^-CD56^{dim}$, and express high levels of perforin and granzyme B, however a subset of $CD27^+$ NK-cells are identified as $CD56^{dim/bright}$ with low levels of perforin and granzyme B [8]. This suggests that, similar to T-cells, the presence or absence of CD27 on NK-cells allows the identification of cytotoxic effector cells within the known mature NK-cell subsets.

Potential for manipulating CD27 co-stimulation for immunotherapy

The potent co-stimulatory capacity of CD27 and its expression across different subsets makes the CD27/CD70 signalling pathway a desirable target for immunotherapy. Different strategies of blocking or augmenting CD27/CD70 co-stimulation and the resultant outcomes for acute or chronic viral infections, autoimmune diseases, tumours, are discussed.

(i) Blocking CD27 co-stimulation may protect against immunopathology during chronic viral infections or autoimmunity.

Although beneficial during acute infections, CD27 co-stimulation may be detrimental during autoimmune or chronic viral infections [10,31] (Table 4). Lymphocytes derived from patients with the autoimmune disease systemic lupus erythematosus (SLE) [54] or rheumatoid arthritis [55] have a ~2-fold increase in CD70 expression on $CD4^+$ T-cells, compared to healthy individuals. Since CD27 co-stimulation influences the production of pro-inflammatory cytokines, it might contribute to the pathology associated with inflammatory autoimmune diseases [10]. Indeed, SJL/J [56], DBA/1 [57] and $RAG^{-/-}$ [58] mice activated to induce disease in the absence of CD27-stimulation had a reduction (~4-times) in clinical scores [9,30], suggesting that CD27 co-stimulation might be detrimental during particular autoimmune diseases, and that blocking CD27 co-stimulation may be a feasible option for future autoimmune immunotherapies (Table 4).

During chronic viral infections, T-cells become "exhausted" [59]. Strikingly, increased expression of CD70 and decreased CD27 expression was observed directly *ex vivo* on naïve $CD19^{+}CD27^{-}$ B-cells [60] and $CD3^{+}$ T-cells [61] isolated from HIV-infected individuals compared to healthy donors, suggesting that prolonged CD27 co-stimulation may contribute to immunopathology during certain chronic infections. Using a transgenic mouse model with constitutive CD70 expression on T-cells to mimic the expression patterns observed in chronic infections, it was found that $CD8^{+}$ T-cells subjected to continuous CD27 co-stimulation displayed enhanced response magnitude to influenza infection compared to WT mice [41]. However, these cells exhibited an exhausted phenotype, as measured by $CD69^{hi}PD\text{-}1^{hi}IL7R^{lo}$ expression, and decreased IL-2/TNF production. Interestingly, CD70 blockade in mice during chronic LCMV (LC-13) infection increased numbers of IFNγ-producing virus-specific $CD8^{+}$ T-cells, but did not alter their exhausted phenotype [40], and accelerated viral clearance [62]. Thus, uncontrolled CD27 co-stimulation deregulates effector T cell differentiation, promotes exhaustion and contributes to the detrimental outcomes of chronic infections [40,62]. CD27 co-stimulation thus requires careful regulation [10]. As such, there is emerging interest in blocking CD27 co-stimulation in chronic viral infections and inflammatory disease [9,31].

(ii) Augmenting CD27 co-stimulation may help in cellular immunity to cancer

CD70 is highly expressed in multiple cancers including thymic carcinoma [38], cultivated brain tumours [63] and renal carcinoma [64]. Hence, CD70 and CD27 are considered as targets for immunotherapy [31,65-68]. Augmenting CD27 co-stimulation through use of CD70-secreting or expressing tumour cells in mice has resulted in potent enhancement of cell-mediated anti-tumour immunity to reduce or prevent tumour development, even at locations distal to the treatment site [69,70] (Table 5). This suggests that augmenting CD27 co-stimulation enhances systemic anti-tumour immunity and thus may be important for future immunotherapies. Indeed, a promising agonist anti-CD27 antibody is currently in phase I clinical trials. This immunotherapy, known as Varlilumab, is manufactured by Celldex Therapeutics and is being trialled against solid tumours and lymphoid malignancies [71] (Table 5). Although preliminary, this trial is highly promising and proves that manipulating CD27 co-stimulation may be an effective and viable anti-tumour immunotherapy, possibly in combination with other immunotherapies such as PD-1 blockade [72**].

Conclusion

CD27 is expressed on the majority of human T-cells and B-cells. Binding of CD27 to its only known ligand, CD70, has potent co-stimulatory effects, which can be either beneficial or detrimental in different circumstances. Due to these potent co-stimulatory effects, there is a great interest in manipulating CD27 co-stimulation for immunotherapy. Blocking CD27 co-stimulation may prevent/reduce the severity of chronic viral infections and autoimmune diseases. Conversely, augmenting CD27 co-stimulation may assist in anti-tumour immunity and may rescue exhausted $CD8^+$ T-cells. Thus, targeting and manipulating the CD27-CD70 immune pathway is an exciting and emerging field, with a great promise for novel immunotherapies.

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Figure Legend

Figure 1. Effects of CD27 co-stimulation on naïve, effector, and memory $CD8^+$ T cells. CD27 co-stimulation of naïve $CD8^+$ T cells via CD70 expressed on DCs or APCs leads to the activation of canonical and non-canonical NF-κB pathways, resulting in up-regulation of anti-apoptotic molecules and cytokine receptors. Augmented CD27 co-stimulation during primary activation increases a number of epitope-specific $CD8^+$ T cells, enhanced effector function and retention of IL-7Rα expression, thus elevating numbers of $CD8^+$ T cells that persist into memory and participate in recall responses. Interestingly, chronic CD27 co-stimulation results in reduced T cell memory and impaired protection against subsequent virus infection.

Diminished CD27 co-stimulation is associated with numerically and functionally reduced $CD8^+$ T cell responses and decreased memory formation. Given these potent effects of CD27 co-stimulation on the magnitude and quality of $CD8^+$ T cell immunity, manipulating CD27 signalling may prove an effective target of immunotherapies not just for viruses, but also chronic diseases and cancer.

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Augmenting CD27 co-stimulation with an agonistic CD27 mAb in the absence of inflammation can rescue exhausted cells.

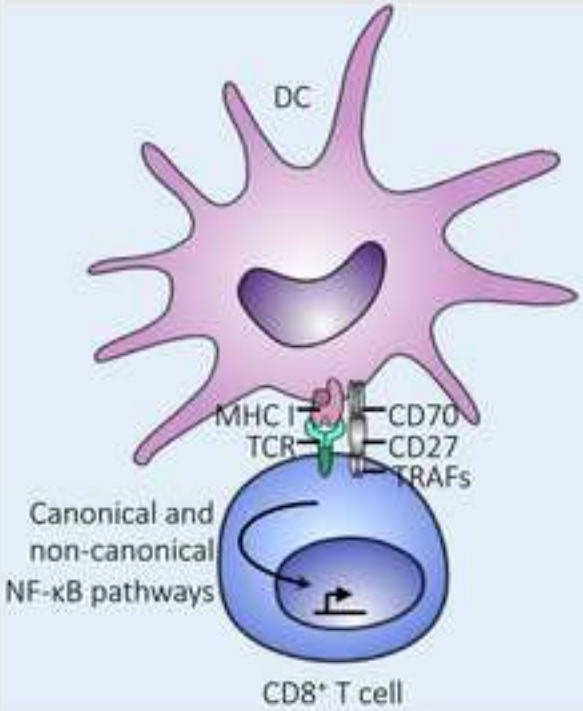
Highlights

- CD27 is a co-stimulatory receptor expressed on T-cells, B-cells and NK-cells
- CD27-CD70 co-stimulation enhances primary, memory and recall T-cell responses
- Manipulating CD27-CD70 signalling is of interest for a variety of immunotherapies

CD27 co-stimulation in CD8⁺ T cell mediated anti-viral immunity

Naïve CD8⁺ T cells

Inducible CD70 expression
(TLR4/9, IFNγ, CD40)

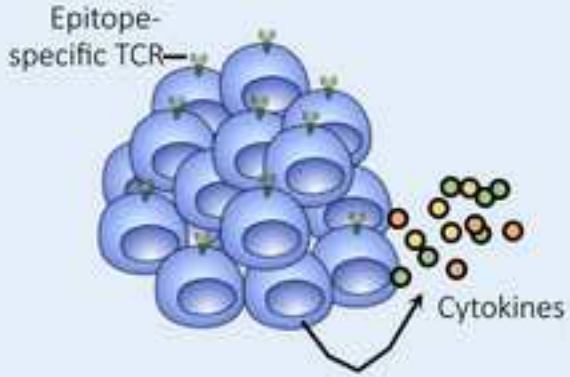


↑ Anti-apoptotic molecules (BCL-XL)
↑ Cytokine receptors (IL-2Rα, IL-12β)

Effector CD8⁺ T cells

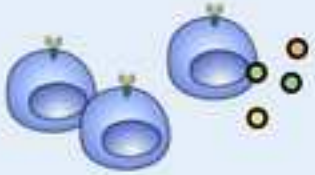
Augmented CD27 stimulation

↑ Expansion of epitope-specific T cells



Diminished CD27 stimulation

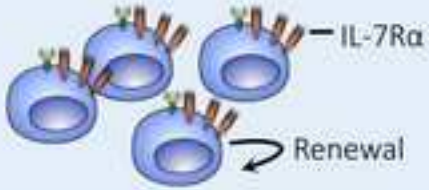
↓ Number of epitope-specific T cells
↓ Function
↓ Proliferation



Memory CD8⁺ T cells

Augmented CD27 stimulation

↑ Number of epitope-specific T cells
↑ Secondary responses
● Retention of IL-7Rα expression



Constitutive CD27 stimulation

↓ Secondary responses
● Impaired protection

Diminished CD27 stimulation

↓ IL-7Rα expression
● Delayed recall
● Early reduction in virus-specific T cells

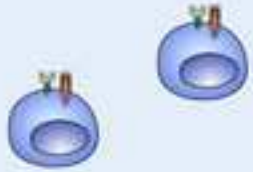


Table 1

Table 1. Publications describing the role of CD27 co-stimulation in mice

Model	Experimental design	Method of detection	Major findings	Key finding	Reference
CD27 co-stimulation is important for T-cell development					
RAG-/-	Block CD27 co-stimulation with a mAb during thymic development	Flow-cytometry	Decreased transition of DN to DP thymocytes	CD27 co-stimulation is needed for transition of DN to DP thymocytes	Gravestein *et al*, 1996 [Ref. 36]
Enhanced CD27 co-stimulation increases primary, memory and recall CD8$^+$ T-cell responses					
OT-1 and C57BL/6	Naïve OT-1 CD8$^+$ T-cells were adoptively transferred into naïve C57BL/6 mice. $OVA_{257-264}$ peptide was administered in the presence or absence of sCD70, or an agonist CD27 mAb	Tetramer & flow-cytometry	~17-fold increase in the proportion of OT-1 CD8$^+$ T-cells 4, 6 and 8dpa	Enhanced CD27 co-stimulation augments primary, memory and recall CD8$^+$ T-cell responses	Rowley *et al*, 2004 [Ref. 11]
		^{51}Cr killing assay	>15-fold increase in cytotoxicity 10dpa		
		Tetramer & flow-cytometry	~36-fold increase in the proportion of OT-1 CD8$^+$ T-cells at memory, 23dpa		
		Tetramer & flow-cytometry	>20-fold increase in OT-1 CD8$^+$ T-cells in the peripheral blood 8dpr and ~12-fold 20dpr		
OT-1 and C57BL/6	Naïve OT-1 CD8$^+$ T-cells were adoptively transferred into naïve C57BL/6mice and activated by OVA peptide in the presence or absence of sCD70	Tetramer & flow-cytometry	>50-fold increase in OT-1 CD8$^+$ T-cells in peripheral blood 8dpa	CD27 co-stimulation enhances primary and memory CD8$^+$ T-cell responses	Willoughby *et al*, 2014 [Ref. 37]
		Tetramer-ICS & flow cytometry	~3-4 fold increase in IL-2$^+$, IFNγ$^+$ and perforin$^+$ OT-1 CD8$^+$ T-cells 3dpa		
		Tetramer & flow-cytometry	~2-fold increase in the number of OT-1 CD8$^+$ T-cells in the peripheral blood 65dpa		
		Tetramer & flow-cytometry	~6-fold increase in IL-7Rα expression on OT-1 CD8$^+$ T-cells 3dpa, but ~2-fold 4dpa	Enhanced CD27 co-stimulation decreases IL7Rα down-regulation	
Transgenic mice with constitutive CD70 expression on T-cells	WT and transgenic mice were infected with IAV	Weight loss	~10% more body weight 8-12dpi	Constitutive CD27 co-stimulation enhances primary CD8$^+$ T cell responses, but results in reduced memory formation	Van Gisbergen *et al*, 2009 [Ref. 41]
		qPCR	~2-log decrease in viral lung titres		
		Tetramer & flow-cytometry	~2-fold increase in NP_{366}-specific CD8$^+$ T-cells in blood, MLN and spleen 10dpi		
		ICS & flow-cytometry	2-fold increase in the number of IFNγ$^+$ T-cells 10dpi		
		Tetramer & flow-cytometry	~4-fold reduction in the number of NP_{366}-specific CD8$^+$ T-cells in the spleen 57dpi		
	WT or transgenic mice were infected with	Tetramer & flow-cytometry	~4-fold decrease in NP_{366}-specific CD8$^+$ T-	Constitutive CD27 co-stimulation during	

	IAV then challenged 51-61 days later with a serologically distinct IAV		cells in the spleen and blood 8dpc	both primary and secondary activation decreases recall	
		ICS and flow-cytometry	~4-fold decrease in the number of IFNγ^+ CD8$^+$ T-cells 8dpc		
CD27$^{-/-}$	CD27$^{-/-}$ and WT mice were infected with IAV	Tetramer & flow-cytometry	~3-fold decrease in total and NP_{336}-specific CD8$^+$ T-cells, in the lung 10dpi	Loss of CD27 co-stimulation decreases CD8$^+$ T-cell proliferation	Hendricks *et al*, 2003 [Ref. 12]
	CD27$^{-/-}$ or WT mice were infected with IAV and challenged 6 weeks later	Tetramer & flow-cytometry	~7-fold and ~14-fold decrease in the number of total or NP_{338}-specific CD8$^+$ T-cells, respectively 5dpc. Decreased to ~1.5-fold 7dpc	Loss of CD27 co-stimulation delays recall during secondary IAV infection	
	Purified T-cells from WT and CD27$^{-/-}$ mice were activated by αCD3 cross-linking +/- additional αCD28 *in vitro*	Thymidine incorporation	~2-fold decrease in proliferation 3dpa for T-cells from CD27$^{-/-}$ mice in the absence of αCD28	CD27 and CD28 co-stimulation are qualitatively different	
			αCD28 increases proliferation of CD8$^+$ T-cells from CD27$^{-/-}$ mice, but not to same extent as WT mice 3dpa		
CD70$^{-/-}$ and C57BL/6	CD70$^{-/-}$ or WT mice were infected with acute LCMV	Tetramers	<2-fold decrease in total and NP_{396}-specific CD8$^+$ T-cells, 6-8dpi	Loss of CD27 co-stimulation decreases epitope-specific proliferation, differentiation and function, and reduces viral clearance	Munitic *et al*, 2013 [Ref. 39]
		Flow cytometry	Decreased differentiation by CD44^{hi} and CD62L expression 8dpi		
		ICS & flow-cytometry	~2-fold reduction in IFNγ, TNF and IL-2 6-8dpi		
		qRT-PCR	~5-fold reduction in viral clearance 6 and 8dpi		
C57BL/6	C57BL/6 mice were infected with acute LCMV in the absence or presence of a CD70 blocking mAb	ICS & flow-cytometry	~6-fold reduction in the proportion and numbers of IFNγ^+ TNF$^+$ cells 7dpi.	Loss of CD27 co-stimulation decreases the function of epitope-specific CD8$^+$ T-cells	Penaloza-McMaster *et al*, 2011 [Ref. 40]
OT-1 And C57BL/6	OT-1 CD8$^+$ T-cells were transferred into C57BL/6 mice. Mice were vaccinated with OVA-vac and treated with or without an αCD70- blocking mAb	Tetramer & flow-cytometry	~5-fold decrease in the number of resting memory OT-1 CD8$^+$ T-cells 90dpa	Blocking CD27 co-stimulation decreases the number of IL-7Rα expressing memory precursor cells	Dong *et al*, 2012 [Ref. 42]
		Flow-cytometry	~2-fold decrease in IL7Rα expression 7dpa		
C57BL/6	C57BL/6 mice were immunised with OVA peptide and an agonistic anti-CD40 antibody and recalled 15-48 days later with OVA peptide, in the absence or presence of a CD27 agonist mAb	Tetramer & flow-cytometry	~8-fold increase in total $OVA_{257-264}$- specific CD8$^+$ T-cells 8dpr	Enhanced CD27 co-stimulation during secondary activation enhances recall	Taraban *et al*, 2013 [Ref. 45]
		^{51}Cr killing assay	~12-fold increase in cytotoxicity 6pdr		

IAV=influenza A virus, dpi=days post infection, dpa=days post activation, dpt=days post transfer, pdc=days post challenge, dpr=days post recall

Table 2

Table 2. Summary of publications describing the role of CD27 co-stimulation on human T-cells

Model	Experimental design	Method of detection	Major findings	Key finding	Reference
CD27 co-stimulation enhances T-cell function in humans					
Human T-cells	PBMCs were negatively enriched for T-cells. Stimulated with a suboptimal dose of ConA in the presence Colo679-CD70$^+$ or Colo679-CD70$^-$ cells	Thymidine incorporation	~2-fold decrease in proliferation	CD27 co-stimulation increases proliferation	Braun-Falco *et al*, 2001 [Ref. 48]
Human T-cells	CD3$^+$ T-cells were activated by αCD3 cross-linking with or without cross-linking CD27 with plate bound Varlilumab	cellTitre-Glo luminescence assay	4-fold increase in proliferation 5pda	Enhanced CD27 co-stimulation increases proliferation and function	Vitale *et al*, 2012 [Ref. 46]
		ICS	~2-fold increase in IFNγ and TNF production 2dpa		
Human T-cells	CD3$^+$ T-cells were activated by αCD3 cross-linking with or without CD27 cross-linking with plate bound Varlilumab	ELISA	~4-fold increase in IFNγ, TNF, IL-2 and IL-13 production 72hpa	Increased CD27 co-stimulation enhances cytokine production	Ramakrishna *et al*, 2015 [Ref. 47]
	CD3$^+$ T-cells cultures were activated with αCD3 cross-lining with irradiated CD70-expressing cells	ELISA	~2-fold increase in IFNγ and IL-13 production 72hpa		
	CD3$^+$ T-cells were activated by αCD3 cross-linking with or without cross-linking CD27 with plate bound Varlilumab	Gene microarray	CD27 co-stimulation resulted in a distinct gene expression profile	CD27 co-stimulation influences gene expression	

dpa=days post activation, hpa=hours post activation

Table 3

Table 3. Publications investigating the influence of CD27 co-stimulation on γδ T-cells

Model	Experimental design	Method of detection	Major findings	Key finding	Reference
Mouse studies					
C57BL/6	Total γδ T-cells from spleen and LN were stimulated with αCD3 supplemented with sCD70	CBA and flow cytometery	Dose dependent increase in survival and expression of pro-inflammatory cytokines	CD27 co-stimulation supported survival and proliferation of γδ T cells	Ribot *et al*, 2010 [Ref. 51]
WT and CD27$^{-/-}$	Mice were infected with murine herpes virus and malaria	ICS	~ 1 to 4 fold increase in proportion IFNγ producing γδ T-cells	Loss of CD27 co-stimulation decreased IFNγ production	
C57BL/6	Thymic and splenic γδ T-cells were isolated from embryonic, newborn and adult C57BL/6 mice	Flow cytometery	90% of γδ thymocytes were CD27^{hi}	CD27 expression defines stable IFNγ-producing and IL-17-producing γδ subsets	Ribot *et al*, 2009 [Ref. 1]
WT, TCR α- and TCRβ-deficient, CD27$^{-/-}$	Peripheral γδ T-cells were isolated	Real time PCR and flow cytometery	Decrease in IFNγ expression levels in peripheral	Loss of CD27 co-stimulation decreases IFNγ expression	
FTOC γδ thymocytes	FTOC γδ thymocytes were treated with sCD70 and immunoglobulin	Real time PCR	Upregulation of IFNγ in CD27-expressing γδ-thymocytes and down-regulation of IL-17 in CD27-negative γδ-thymocytes	Enhanced CD27 co-stimulation affected IFN-γ and IL-17 expression	
Human studies					
γ9δ2 T-cell line	γ9δ2 cells were enriched from phosphoantigen expanded PBMCs. MACS-sorted γδ T-cells were stimulated with phosphoantigen in the presence of sCD70 or αCD70	CFSE and CBA	Augmented proliferation and increase in Th1 effector functions	Enhanced CD27 co-stimulation increases proliferation, survival and cytokine production	DeBarros *et al*, 2011 [Ref. 52]

Table 4

Table 4. Publications blocking CD27 co-stimulation for immunotherapy in mice

Model	Experimental design	Method of detection	Major findings	Key finding	Reference
Blocking CD27 co-stimulation protects against autoimmunity					
SJL/J	SJL/J mice were injected with PLP_{139} to initiate experimental autoimmune encephalomyelitis (EAE) in the presence or absence of αCD70 blocking mAb	Activity score	~3-fold decrease in mean clinical score up to 50dpi	Early blocking CD27 co-stimulation reduces EAE a murine model of multiple sclerosis	Nakajima *et al*, 2000 [Ref. 56]
DBA/1	DBA/1 mice were injected with Bovine CII in CFA on day 0 and 21 to initiate murine induced collagen arthritis in the presence of an αCD70 blocking antibody from day 21	Clinical score	~3-fold reduction in clinical score up to 25 days post treatment	Blocking CD27 co-stimulation reduces induced collagen arthritis in a murine model	Oflazoglu *et al*, 2009 [Ref. 57]
		Histopathology vscore	~1.5-fold reduction in histopathology score		
C57BL/6 and $RAG^{-/-}$	$CD4^+CD45RB^{hi}$ naïve T-cells from C57BL/6 mice were transferred into $RAG^{-/-}$ mice to initiate experimental colitis with or without αCD70 blocking mAb (pre-symptomatic)	Activity index	~3-fold reduction in disease severity 8wpa	Blocking CD27 co-stimulation prevents establishment and reduces severity of experimental colitis in a murine model of inflammatory bowel disease	Manocha *et al*, 2009 [Ref. 58]
		Histology	~2-fold decrease in tissue destruction (histology score) 8wpa		
	Experimental colitis was established and mice were treated with or without αCD70 blocking mAb 5 weeks post transfer (post-symptomatic)	Activity index	~2-fold reduction in disease severity 8wpa weeks post activation		
		Histology	~1.5-fold reduction in disease severity 8wpa		
Blocking CD27 co-stimulation protects against chronic viral infection					
Transgenic mice with constitutive CD70 expression on T cells	Naïve mice were assessed at 8 weeks of age	Flow-cytometry	~5-fold increase in $CD8^+$ TEM T-cells in the spleen with a more exhausted phenotype including increased CD69 and PD-1 expression, and decreased IL7Rα expression	Constitutive CD27 co-stimulation deregulates differentiation	Van Gisbergen *et al*, 2009 [Ref. 41]
	TEM cells isolated at 30 weeks of age stimulated with PMA/Ionomycin	ICS & flow-cytometry	~2-fold decrease in polyfunctionality ($IL2^+TNF\alpha^+$) of $CD8^+$ T-cells following restimulation		
C57BL/6	Mice were infected with chronic LCMV in the presence or absence of an αCD70 blocking mAb	ICS & flow-cytometry	~1.5-fold increase in the number of IFNγ expressing cells following restimulation with peptide in an ICS assay 21dpi. <1-fold change 7dpi.	Blocking CD27 co-stimulation increases epitope-specific $CD8^+$ T-cell numbers	Penaloza-McMaster *et al*, 2011 [Ref. 40]
C57BL/6	Mice were infected with a chronic strain of LCMV in the presence or absence of an αCD70 blocking mAb	Immunological focus assay	~1.5-log decrease in viral titres 66dpi	Blocking CD27 co-stimulation increases viral clearance	Matter *et al*, 2006 [Ref. 62]

dpi=days post infection, dpa=days post activation, dpt=days post transfer, pdc=days post challenge, wpi=weeks post infection, wpa=weeks post activation, TEM=effector memory T-cells

Table 5

Table 5. Publications augmenting CD27 co-stimulation for immunotherapy

Model	Experimental design	Method of detection	Major findings	Key finding	Reference
Augmenting CD27 co-stimulation can rescue exhausted cells					
C57BL/6	TCR-transgenic Mh $CD8^+$ T-cells were adoptively transferred into male MHC-matched bone marrow transfer recipients in the presence or absence of an agonistic CD27 mAb	Thymidine incorporation	~2-fold increase in Mh $CD8^+$ T-cell proliferation	Augmenting CD27 co-stimulation in the absence of inflammation can rescue exhausted cells	Buchan *et al*, 2015 [Ref. 72]
	Additional blockade of PD-1	ICS & flow-cytometry	~2-fold increase in the proportion of $IFN\gamma^+$ Mh $CD8^+$ T-cells following restimulation with the cognate UTY peptide		
Augmented CD27 co-stimulation enhances anti-tumour immunity in mice					
BalbC	Mice were injected with live tumour-inducing TSA-WT in combination with irradiated (non-tumour inducing) transfected (mock or CD70-secreting) TSA or MC57 cells	Observation	~20% increase in non-tumour development 27dpi and ~1.5-fold reduction in tumour size 28dpi	Enhancing CD27 co-stimulation increases tumour immunogenicity	Cormary *et al*, 2004 [Ref. 69]
C57BL/6	Mice were injected with tumour-inducing MC38 cells that were uninfected or infected with VV-WT or VV-$CD70^+$	Observation	Complete protection against tumour development 28dpt	Augmented CD27 co-stimulation prevents tumour formation	Lorenz *et al*, 1999 [Ref. 70]
	Mice were vaccinated with HBSS or MC38 cells infected with VV-$CD70^+$. Mice were challenged with uninfected MC38 cells on the opposite flank		~8-fold reduction in tumour volume on the opposite flank 14dpc		
Augmented CD27 co-stimulation increases anti-tumour immunity in humans					
Humans in a clinical trial treated with Varlilumab, an αCD27 agonist mAb	Humans with non-Hodgkin's lymphoma (n=3)	Observation	100% effective, 1 patient partial response, 2 patients stable disease	Enhanced CD27 co-stimulation can reduce tumours in humans	Varlilumab [Ref. 71]
	Humans with renal carcinoma (n=15)		40% effective, 1 individual partial response, 3 experienced stable disease		
	Humans with solid tumours (n=25)		16% effective, 4 patients stable disease		
	Humans with melanomas (n=16)		25% effective, I patient patrial response, 3 individuals stable disease		

dpi=days post infection, dpa=days post activation, dpt=days post transfer, pdc=days post challenge