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

Emma J. Grant^{1,2*}, Simone Nüssing^{1*}, Sneha Sant¹, E Bridie Clemens^{1#} and Katherine Kedzierska^{1#}

¹Department of Microbiology and Immunology, at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne 3000, VIC, Australia; ²Institute of Infection and Immunity, Cardiff University School of Medicine, Heath Park, Cardiff CF14 4XN, United Kingdom; *equal contribution; #equal contribution.

To whom correspondence should be addressed:

#Katherine Kedzierska, kkedz@unimelb.edu.au, Department of Microbiology and Immunology, University of Melbourne, Vic 3010, Australia, Ph: (613) 8344 7962.

Abstract

CD27 is a co-stimulatory immune-checkpoint receptor, constitutively expressed on a broad range of T-cells ($\alpha\beta$ and $\gamma\delta$), 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 ($\alpha\beta$ and $\gamma\delta$) [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 NF κ B 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 pMHC 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

1 cascade of signalling pathways, culminating in the activation and translocation of
2 NFκB to the nucleus of T-cells [25]. This induces T-cell proliferation and
3 differentiation, resulting in the acquisition of effector functions and modification of
4 cell surface markers, including cytokine/chemokine receptors and integrins that
5 enable migration to the site of infection [26].
6
7
8
9

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

11 While studies to date have focused predominantly on co-stimulation via CD28, more
12 recently the role of CD27 co-stimulation in T-cell activation has been acknowledged
13 [9,13]. CD27, first characterised by van Lier *et al* [27] in 1987, is a co-stimulatory
14 molecule in both mice [28] and humans [27,29]. It is a transmembrane homodimer of
15 the TNFR family [9,30,31], constitutively expressed on the surface of progenitor and
16 naïve T-cells, as well as subsets of NK- and B-cells [9]. Its ligand, CD70, is inducible
17 on APCs, DCs [32], B cells (triggered by TLR4/9, IFNγ and CD40) and T-cells
18 (following TCR interactions in the presence of CD28 cross-linking) and is
19 constitutively expressed on smooth muscle cells [9]. Following the interaction
20 between CD27 and CD70, TNFR-associated factor (TRAFs) adaptors are recruited
21 [33], which then activate CD8⁺ T-cells through both canonical and alternative NFκB
22 pathways [9,10]. The CD27-CD70 interaction also induces the up-regulation of anti-
23 apoptotic molecules (BCL-XL) [34] and cytokine receptors (IL-2Rα and IL-12Rβ),
24 thus increasing CD8⁺ T-cell sensitivity to cytokines [9]. This interaction facilitates
25 activation of Jun N-terminal kinase (JNK), activator protein 1 (Ap1), eRK and
26 mitogen activated protein (MAP) kinases to promote cytokine production including:
27 IL-2, IL-4, IL-5, IL-6, IL-12, IFNγ and TNFα [9]. CD27 expression on T-cells
28 increases following activation and is accompanied by release of a soluble extracellular
29 part of the molecule [35]. Loss of CD27 expression on T-cells is observed during
30 prolonged stimulation and is associated with fully a differentiated effector phenotype.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50

51 **CD27 co-stimulation enhances antiviral T-cell immunity**

52 Analyses in mice suggest that co-stimulation through CD27 is important during T-cell
53 development [36], primary activation [11,37*-41], transition into memory
54 [11,37*,42], secondary recall and the long-term survival of T-cells [13,31]
55 (summarised in Figure 1 and Table 1). Gravestien *et al* [36] observed CD27
56
57
58
59
60
61
62
63
64
65

1 expression on thymocytes during the double negative (DN) stage of development and
2 using RAG^{-/-} mice showed that blocking CD27 co-stimulation with a mAb decreased
3 the transition of DN to double positive (DP) thymocytes, thus revealing that CD27 co-
4 stimulation is important in T-cell development.
5
6
7

8
9 (i) CD27 co-stimulation enhances primary anti-viral CD8⁺ T-cell responses

10 Published evidence reveals the importance of CD27 co-stimulation during primary
11 viral infection (Table 1). Willoughby *et al* [37*] and Rowley *et al* [11] adoptively
12 transferred OT-1 CD8⁺ T-cells into naïve mice and activated them with OVA peptide
13 in the presence of a CD27-agonist antibody. Augmented CD27 co-stimulation
14 increased the expansion of epitope-specific CD8⁺ T-cells ~50-fold [37*], improved
15 effector function and enhanced cytotoxicity in response to re-stimulation with peptide
16 [11]. Similarly, using CD70-transgenic mice with constitutive CD70 expression on T-
17 cells [41], CD27 co-stimulation resulted in a ~2-fold increase in the number and
18 function of D^bNP₃₆₆⁺CD8⁺ T-cells and accelerated viral clearance following influenza
19 A virus (IAV) infection. Conversely, in CD27^{-/-} mice, the number of total
20 D^bNP₃₆₆⁺CD8⁺ T-cells was decreased in the lungs at 10 days after IAV infection [38].
21 Furthermore, T-cells isolated from CD27^{-/-} mice were less likely to proliferate
22 compared to T-cells isolated from wild-type (WT) mice following anti-CD3 cross-
23 linking *in vitro*. Interestingly, anti-CD28 co-stimulation augmented this proliferation,
24 but not to WT levels, suggesting that CD27 and CD28 co-stimulation are not
25 redundant and are qualitatively different. CD70^{-/-} mice infected with acute LCMV
26 displayed a <2-fold decrease in total D^bNP₃₉₆⁺CD8⁺ T-cells 6-8 days post infection
27 (dpi) and a ~5-fold reduction in viral clearance compared to WT mice [39*].
28 Similarly, CD8⁺ T-cells from WT mice infected with acute LCMV and treated with a
29 blocking CD70 mAb [40] were less functional upon peptide re-simulation at 7dpi.
30 Collectively, these studies demonstrate that CD27 co-stimulation is important for
31 CD8⁺ T-cell proliferation, cytotoxicity and function and enhances viral clearance
32 during primary infection with acute IAV and LCMV.
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

53
54 (ii) CD27 co-stimulation augments memory CD8⁺ T-cell pools

55 CD27 co-stimulation also increases the magnitude of memory epitope-specific CD8⁺
56 T-cell populations. In adoptive transfer experiments with OT-1 CD8⁺ T-cells,
57
58
59
60
61
62
63
64
65

1 augmenting CD27 co-stimulation during priming increased the proportion of OT-1
2 CD8⁺ T-cells >30-fold at 23 days post-activation (dpa) [11]. Conversely, chronic
3 CD27 co-stimulation decreased NP₃₆₆⁺CD8⁺ T-cell numbers 4-fold 57dpi with IAV
4 [41]. The increase in epitope-specific memory with CD27 co-stimulation is likely to
5 result from enhanced IL-7 signalling, as augmentation of CD27 co-stimulation retains
6 IL-7Rα expression on T cells [37*], while blocking CD27 co-stimulation decreases
7 IL-7Rα expression [42]. IL-7, produced by non-hematopoietic cells (e.g. stromal and
8 epithelial cells) and immune cells such as DCs (reviewed in [43]) is functionally
9 important for memory cell development and survival [44]. In this way, CD27 co-
10 stimulation increases sensitivity to IL-7 via IL-7Rα expression and enhances epitope-
11 specific CD8⁺ T-cell transition into memory.
12
13
14
15
16
17
18
19
20
21

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

23 CD27 co-stimulation also enhances CD8⁺ T-cell recall (Table 1). Blocking CD27 co-
24 stimulation in CD27^{-/-} mice delayed CD8⁺ T-cell recall following secondary IAV
25 infection, with an early reduction in virus-specific CD8⁺ T-cells observed 5dpi [38].
26 However, this difference was reduced by 7dpi. Conversely, augmenting CD27 co-
27 stimulation either during priming [11] or recall [45*] enhanced secondary responses
28 by OT-I CD8⁺ T cells to OVA peptide. Interestingly, constitutive CD27 co-
29 stimulation resulted in diminished T cell responses and impaired protection following
30 secondary challenge with IAV [41]. These data show that enhanced, but not
31 constitutive, CD27 co-stimulation during either primary or secondary infection can
32 augment memory formation and recall responses.
33
34
35
36
37
38
39
40
41
42

43 (iv) Effects of CD27 co-stimulation in humans

44 Despite numerous murine studies, little is known about the role of CD27 co-
45 stimulation in human T-cells (Table 2). *In vitro* findings show that augmenting CD27
46 co-stimulation by CD27 cross-linking [46,47**] or the addition of Colo679-CD70-
47 expressing cells [48] increased proliferation and function (IFNγ/TNF expression) of
48 human T-cells 2- to 4-fold following non-specific activation [46,48]. Expectedly,
49 gene expression profiling showed activation and proliferation profiles in T-cells with
50 enhanced CD27 co-stimulation [47**]. Two independent studies also correlated the
51 loss of CD27 co-stimulation with disease severity. A total of 8 patients with severe
52 infectious mononucleosis (IM) and complications including EBV-associated
53
54
55
56
57
58
59
60
61
62
63
64
65

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 $\gamma\delta$ T-cells, B-cells and NK-cells

(i) Expression of CD27 on murine and human $\gamma\delta$ T-cells

Recently, it became apparent that two functionally distinct subsets of $\gamma\delta$ T-cells display differential expression of CD27. Although both CD27⁺ and CD27⁻ subtypes produce IFN- γ , only CD27⁻ $\gamma\delta$ 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 $\gamma\delta$ T-cells was characterised on thymocytes derived from foetal organ thymic cultures (FTOC) and showed that CD27⁺ $\gamma\delta$ thymocytes had higher *Ifng* mRNA expression, while CD27⁻ $\gamma\delta$ thymocytes had decreased *Il17* expression (Table 3). Furthermore, CD27⁻ $\gamma\delta$ T-cells isolated from the spleen, lymph nodes (LN), lung or gut were CD44^{hi} and CD62L^{lo}, whereas CD27⁺ $\gamma\delta$ T-cells had lower CD44 expression, supporting CD27 as a marker of $\gamma\delta$ T-cell differentiation with distinct functional outcomes [1]. Lombes *et al* [3*] reported that innate-like IL-17-producing CD27⁻ $\gamma\delta$ T-cells correspond to Ly-6C⁻CD44^{hi} $\gamma\delta$ 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 $\gamma\delta$ T-cells remains largely unknown. Lombes *et al* [3*] suggested that peripheral CD27⁺ $\gamma\delta$ T-cells are similar to $\alpha\beta$ 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⁺ $\gamma\delta$ T-cells in a similar manner. This was addressed by Ribot and colleagues [51], who stimulated murine CD27⁺ $\gamma\delta$ T-cells *in vitro* with sCD70 and showed increased IFN γ /TNF production with CD27 co-stimulation. Additionally, accumulation of IFN γ -producing CD27⁺ $\gamma\delta$ 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 $\gamma\delta$ T-cells. deBarros *et al* [52] showed that

1 interaction of CD27 with its ligand CD70 resulted in increased TCR-dependent
2 activation in $\gamma\delta$ T-cell lines, increased proliferation, enhanced survival and cytokine
3 production, establishing the importance of CD27 co-stimulation on the functional
4 differentiation of human $\gamma\delta$ T-cells (Table 3). Since $\gamma\delta$ T-cells contribute to anti-
5 viral and anti-cancer immunity, it is important to elucidate the role of CD27 co-
6 stimulation and its potential role for future immunotherapies.
7
8
9

10 11 12 *(ii) CD27 as a memory B-cell marker*

13 Like T-cells, human B-cells can be subdivided according to their CD27 expression.
14 However, unlike T-cells, naïve B-cells do not express CD27 and instead expression of
15 CD27 is associated with memory. Interestingly, CD27⁻ B-cells populate the non-
16 mutated V gene compartment of naïve-like B-cells [4], while expression of CD27
17 (CD27⁺) is commonly used to identify human memory B-cells with mutated V genes
18 [4,5]. Circulating CD27⁺ memory B-cells can be further subdivided by their relative
19 expression of the immunoglobulin (Ig) antibodies IgM and IgD [4,53]. Up to 40% of
20 human peripheral B-cells express CD27 and show mutated variable regions in their Ig
21 genes [6], making the CD27 receptor an interesting marker for B-cell subsetting.
22 However, a minor CD27⁻ memory B-cell subset makes up 1-4% of all peripheral B-
23 cells [6]. Therefore, CD27 expression alone is insufficient for B-cell memory
24 identification. The function of CD27 co-stimulation on human memory B-cells has
25 not been characterised, however, it is thought that, similar to T-cells, CD27-receptor
26 signalling in B-cells can enhance survival [12]. It would be interesting to understand
27 whether CD27 co-stimulation on B-cells is beneficial for maintaining lifelong
28 serological memory by promoting the survival of memory B-cells. As such,
29 determining the functional role of CD27 co-stimulation for B-cells may provide
30 further potential for CD27-based immunotherapies.
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49

50 *(iii) CD27 expression marks functionally distinct NK-cells*

51 NK-cells can also be sub-divided based on CD27 expression in both mice and humans
52 [7,8]. In mice, the presence or absence of CD27 expression results in distinct effector
53 functions, proliferative capacities, responsiveness, interaction with DCs, and
54 migratory activity of NK-cells [7]. Mac1^{high}CD27⁺ murine NK-cells show increased
55 IFN γ production compared to CD27⁻ NK-cells following activation with the NKG2D
56
57
58
59
60
61
62
63
64
65

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-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 IFN γ -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.

Acknowledgements

This work was supported by Australian National Health and Medical Research Council (NHMRC) Program (AI1071916) Grant to KK. EJG is a recipient of a NHMRC CJ Martin Fellow, EBD is a NHMRC Peter Doherty Fellow and KK is an NHMRC SRF Level B. SN was supported by a Melbourne International Fee Remission Scholarship (MIFRS) and Melbourne International Research Scholarship (MIRS). SS was supported by a Victoria India Doctoral Scholarship (VIDS) and MIFRS.

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.

References

1. Ribot JC, deBarros A, Pang DJ, Neves JF, Peperzak V, Roberts SJ, Girardi M, Borst J, Hayday AC, Pennington DJ, et al.: **CD27 is a thymic determinant of the balance between interferon-gamma-and interleukin 17-producing gamma delta T cell subsets.** *Nature Immunology* 2009, **10**:427-436.
2. Taghon T, Yui MA, Pant R, Diamond RA, Rothenberg EV: **Developmental and molecular characterization of emerging beta- and gamma delta-selected pre-T cells in the adult mouse thymus.** *Immunity* 2006, **24**:53-64.
The authors show that the phenotypic, functional, and homeostatic characteristics of the CD27⁺ γδ T cell compartment is comparable to that of naive and memory CD8⁺ αβ T cells, unlike the CD27⁻ γδ T cell compartment in mice.
- * 3. Lombes A, Durand A, Charvet C, Riviere M, Bonilla N, Auffray C, Lucas B, Martin B: **Adaptive Immune-like gamma/delta T Lymphocytes Share Many Common Features with Their alpha/beta T Cell Counterparts.** *Journal of Immunology* 2015, **195**:1449-1458.
The authors show that the phenotypic, functional, and homeostatic characteristics of the CD27⁺ γδ T cell compartment is comparable to that of naive and memory CD8⁺ αβ T cells, unlike the CD27⁻ γδ T cell compartment in mice.
4. Klein U, Rajewsky K, Kuppers R: **Human immunoglobulin (Ig)M(+)IgD(+) peripheral blood B cells expressing the CD27 cell surface antigen carry somatically mutated variable region genes: CD27 as a general marker for somatically mutated (memory) B cells.** *Journal of Experimental Medicine* 1998, **188**:1679-1689.
5. Tangye SG, Liu YJ, Aversa G, Phillips JH, de Vries JE: **Identification of functional human splenic memory B cells by expression of CD148 and CD27.** *Journal of Experimental Medicine* 1998, **188**:1691-1703.
6. Fecteau JF, Cote G, Neron S: **A new memory CD27(-)IgG(+) B cell population in peripheral blood expressing V-H genes with low frequency of somatic mutation.** *Journal of Immunology* 2006, **177**:3728-3736.
7. Hayakawa Y, Smyth MJ: **CD27 dissects mature NK cells into two subsets with distinct responsiveness and migratory capacity.** *Journal of Immunology* 2006, **176**:1517-1524.
8. Vossen MTM, Matmati M, Hertoghs KML, Baars PA, Gent NR, Leclercq G, Hamann J, Kuijpers TW, van Lier RAW: **CD27 defines phenotypically and functionally different human NK cell subsets.** *Journal of Immunology* 2008, **180**:3739-3745.
9. Croft M: **The role of TNF superfamily members in T-cell function and diseases.** *Nat Rev Immunol* 2009, **9**:271-285.

10. Nolte MA, van Olfen RW, van Gisbergen KP, van Lier RA: **Timing and tuning of CD27-CD70 interactions: the impact of signal strength in setting the balance between adaptive responses and immunopathology.** *Immunol Rev* 2009, **229**:216-231.
11. Rowley TF, Al-Shamkhani A: **Stimulation by soluble CD70 promotes strong primary and secondary CD8+ cytotoxic T cell responses in vivo.** *J Immunol* 2004, **172**:6039-6046.
12. Hendricks J, Xiao YL, Borst J: **CD27 promotes survival of activated T cells and complements CD28 in generation and establishment of the effector T cell pool.** *Journal of Experimental Medicine* 2003, **198**:1369-1380.
13. Dolfi DV, Katsikis PD: **CD28 and CD27 costimulation of CD8+ T cells: a story of survival.** *Adv Exp Med Biol* 2007, **590**:149-170.
14. Banchereau J, Steinman RM: **Dendritic cells and the control of immunity.** *Nature* 1998, **392**:245-252.
15. Bennett SRM, Carbone FR, Karamalis F, Miller JFAP, Heath WR: **Induction of a CD8(+) cytotoxic T lymphocyte response by cross-priming requires cognate CD4(+) T cell help.** *Journal of Experimental Medicine* 1997, **186**:65-70.
16. Ridge JP, Di Rosa F, Matzinger P: **A conditioned dendritic cell can be a temporal bridge between a CD4+ T-helper and a T-killer cell.** *Nature* 1998, **393**:474-478.
17. Bedoui S, Heath WR, Mueller SN: **CD4(+) T-cell help amplifies innate signals for primary CD8(+) T-cell immunity.** *Immunological Reviews* 2016, **272**:52-64.
18. Mescher MF, Curtsinger JM, Agarwal P, Casey KA, Gerner M, Hammerbeck CD, Popescu F, Xiao Z: **Signals required for programming effector and memory development by CD8+ T cells.** *Immunol Rev* 2006, **211**:81-92.
19. den Haan JM, Arens R, van Zelm MC: **The activation of the adaptive immune system: cross-talk between antigen-presenting cells, T cells and B cells.** *Immunol Lett* 2014, **162**:103-112.
20. Hennecke J, Wiley DC: **T cell receptor-MHC interactions up close.** *Cell* 2001, **104**:1-4.
21. Watts TH: **Tnf/tnfr family members in costimulation of T cell responses.** *Annual Review of Immunology* 2005, **23**:23-68.
22. Esensten JH, Helou YA, Chopra G, Weiss A, Bluestone JA: **CD28 Costimulation: From Mechanism to Therapy.** *Immunity* 2016, **44**:973-988.
23. June CH, Bluestone JA, Nadler LM, Thompson CB: **The B7 and CD28 receptor families.** *Immunol Today* 1994, **15**:321-331.
24. Curtsinger JM, Schmidt CS, Mondino A, Lins DC, Kedl RM, Jenkins MK, Mescher MF: **Inflammatory cytokines provide a third signal for activation of naive CD4+ and CD8+ T cells.** *J Immunol* 1999, **162**:3256-3262.
25. Smith-Garvin JE, Koretzky GA, Jordan MS: **T Cell Activation.** *Annual Review of Immunology* 2009, **27**:591-619.
26. Butcher EC, Picker LJ: **Lymphocyte homing and homeostasis.** *Science* 1996, **272**:60-66.
27. van Lier RA, Borst J, Vroom TM, Klein H, Van Mourik P, Zeijlemaker WP, Melief CJ: **Tissue distribution and biochemical and functional properties of Tp55 (CD27), a novel T cell differentiation antigen.** *J Immunol* 1987, **139**:1589-1596.

28. Gravestein LA, Nieland JD, Kruisbeek AM, Borst J: **Novel mAbs reveal potent co-stimulatory activity of murine CD27.** *Int Immunol* 1995, **7**:551-557.
 29. Kobata T, Jacquot S, Kozlowski S, Agematsu K, Schlossman SF, Morimoto C: **CD27-CD70 interactions regulate B-cell activation by T cells.** *Proc Natl Acad Sci U S A* 1995, **92**:11249-11253.
 30. Denoeud J, Moser M: **Role of CD27/CD70 pathway of activation in immunity and tolerance.** *J Leukoc Biol* 2011, **89**:195-203.
 31. Wajant H: **Therapeutic targeting of CD70 and CD27.** *Expert Opinion on Therapeutic Targets* 2016, **20**:959-973.
 32. Sanchez PJ, McWilliams JA, Haluszczak C, Yagita H, Kedl RM: **Combined TLR/CD40 stimulation mediates potent cellular immunity by regulating dendritic cell expression of CD70 in vivo.** *J Immunol* 2007, **178**:1564-1572.
 33. Akiba H, Nakano H, Nishinaka S, Shindo M, Kobata T, Atsuta M, Morimoto C, Ware CF, Malinin NL, Wallach D, et al.: **CD27, a member of the tumor necrosis factor receptor superfamily, activates NF-kappaB and stress-activated protein kinase/c-Jun N-terminal kinase via TRAF2, TRAF5, and NF-kappaB-inducing kinase.** *J Biol Chem* 1998, **273**:13353-13358.
 34. van Oosterwijk MF, Juwana H, Arens R, Tesselaar K, van Oers MH, Eldering E, van Lier RA: **CD27-CD70 interactions sensitise naive CD4+ T cells for IL-12-induced Th1 cell development.** *Int Immunol* 2007, **19**:713-718.
 35. Hintzen RQ, de Jong R, Lens SM, Brouwer M, Baars P, van Lier RA: **Regulation of CD27 expression on subsets of mature T-lymphocytes.** *J Immunol* 1993, **151**:2426-2435.
 36. Gravestein LA, van Ewijk W, Ossendorp F, Borst J: **CD27 cooperates with the pre-T cell receptor in the regulation of murine T cell development.** *J Exp Med* 1996, **184**:675-685.
 - *37. Willoughby JE, Kerr JP, Rogel A, Taraban VY, Buchan SL, Johnson PW, Al-Shamkhani A: **Differential impact of CD27 and 4-1BB costimulation on effector and memory CD8 T cell generation following peptide immunization.** *J Immunol* 2014, **193**:244-251.
- CD27 co-stimulation via the addition of sCD70 enhances primary and memory CD8⁺ T-cell responses and promotes retention of IL-7R α .
38. Hendriks J, Gravestein LA, Tesselaar K, van Lier RA, Schumacher TN, Borst J: **CD27 is required for generation and long-term maintenance of T cell immunity.** *Nat Immunol* 2000, **1**:433-440.
 - *39. Munitic I, Kuka M, Allam A, Scoville JP, Ashwell JD: **CD70 deficiency impairs effector CD8 T cell generation and viral clearance but is dispensable for the recall response to lymphocytic choriomeningitis virus.** *J Immunol* 2013, **190**:1169-1179.
- Loss of CD27 co-stimulation in CD70^{-/-} mice decreases CD8⁺ T cell proliferation, differentiation and function, and reduces viral clearance during CMV infection.
40. Penaloza-MacMaster P, Ur Rasheed A, Iyer SS, Yagita H, Blazar BR, Ahmed R: **Opposing effects of CD70 costimulation during acute and chronic lymphocytic choriomeningitis virus infection of mice.** *J Virol* 2011, **85**:6168-6174.
 41. van Gisbergen KP, van Olffen RW, van Beek J, van der Sluijs KF, Arens R, Nolte MA, van Lier RA: **Protective CD8 T cell memory is impaired during chronic CD70-driven costimulation.** *J Immunol* 2009, **182**:5352-5362.

42. Dong H, Franklin NA, Roberts DJ, Yagita H, Glennie MJ, Bullock TN: **CD27 stimulation promotes the frequency of IL-7 receptor-expressing memory precursors and prevents IL-12-mediated loss of CD8(+) T cell memory in the absence of CD4(+) T cell help.** *J Immunol* 2012, **188**:3829-3838.
 43. Gao J, Zhao L, Wan YY, Zhu B: **Mechanism of Action of IL-7 and Its Potential Applications and Limitations in Cancer Immunotherapy.** *Int J Mol Sci* 2015, **16**:10267-10280.
 44. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R: **Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells.** *Nat Immunol* 2003, **4**:1191-1198.
 - *45. Taraban VY, Rowley TF, Kerr JP, Willoughby JE, Johnson PM, Al-Shamkhani A, Buchan SL: **CD27 costimulation contributes substantially to the expansion of functional memory CD8(+) T cells after peptide immunization.** *Eur J Immunol* 2013, **43**:3314-3323.
- Enhancing CD27 co-stimulation with a CD27 agonistic mAb during secondary activation enhances recall.
46. Vitale LA, He LZ, Thomas LJ, Widger J, Weidlick J, Crocker A, O'Neill T, Storey J, Glennie MJ, Grote DM, et al.: **Development of a human monoclonal antibody for potential therapy of CD27-expressing lymphoma and leukemia.** *Clin Cancer Res* 2012, **18**:3812-3821.
 - **47. Ramakrishna V, Sundarapandiyam K, Zhao B, Bylesjo M, Marsh HC, Keler T: **Characterization of the human T cell response to in vitro CD27 costimulation with varlilumab.** *J Immunother Cancer* 2015, **3**:37.
- Increased CD27 co-stimulation with a novel anti-CD27 antibody named Varlilumab enhances cytokine production and can influence gene expression following polyclonal activation of human T cells by CD3 cross-linking.
48. Braun-Falco M, Hallek M: **Recombinant adeno-associated virus (rAAV) vector-mediated cotransduction of CD70 and CD80 into human malignant melanoma cells results in an additive T-cell response.** *Arch Dermatol Res* 2001, **293**:12-17.
 49. Salzer E, Daschkey S, Choo S, Gombert M, Santos-Valente E, Ginzl S, Schwendinger M, Haas OA, Fritsch G, Pickl WF, et al.: **Combined immunodeficiency with life-threatening EBV-associated lymphoproliferative disorder in patients lacking functional CD27.** *Haematologica* 2013, **98**:473-478.
 50. van Montfrans JM, Hoepelman AI, Otto S, van Gijn M, van de Corput L, de Weger RA, Monaco-Shawver L, Banerjee PP, Sanders EA, Jol-van der Zijde CM, et al.: **CD27 deficiency is associated with combined immunodeficiency and persistent symptomatic EBV viremia.** *J Allergy Clin Immunol* 2012, **129**:787-793 e786.
 51. Ribot JC, Chaves-Ferreira M, d'Orey F, Wencker M, Goncalves-Sousa N, Decalf J, Simas JP, Hayday AC, Silva-Santos B: **Cutting Edge: Adaptive Versus Innate Receptor Signals Selectively Control the Pool Sizes of Murine IFN-gamma- or IL-17-Producing gamma delta T Cells upon Infection.** *Journal of Immunology* 2010, **185**:6421-6425.
 52. DeBarros A, Chaves-Ferreira M, d'Orey F, Ribot JC, Silva-Santos B: **CD70-CD27 interactions provide survival and proliferative signals that regulate**

- T cell receptor-driven activation of human gammadelta peripheral blood lymphocytes.** *Eur J Immunol* 2011, **41**:195-201.
53. Kruetzmann S, Rosado MM, Weber H, Germing U, Tournilhac O, Peter HH, Berner R, Peters A, Boehm T, Plebani A, et al.: **Human immunoglobulin M memory B cells controlling Streptococcus pneumoniae infections are generated in the spleen.** *Journal of Experimental Medicine* 2003, **197**:939-945.
 54. Han BK, White AM, Dao KH, Karp DR, Wakeland EK, Davis LS: **Increased prevalence of activated CD70+CD4+ T cells in the periphery of patients with systemic lupus erythematosus.** *Lupus* 2005, **14**:598-606.
 55. Lee WW, Yang ZZ, Li G, Weyand CM, Goronzy JJ: **Unchecked CD70 expression on T cells lowers threshold for T cell activation in rheumatoid arthritis.** *J Immunol* 2007, **179**:2609-2615.
 56. Nakajima A, Oshima H, Nohara C, Morimoto S, Yoshino S, Kobata T, Yagita H, Okumura K: **Involvement of CD70-CD27 interactions in the induction of experimental autoimmune encephalomyelitis.** *J Neuroimmunol* 2000, **109**:188-196.
 57. Oflazoglu E, Boursalian TE, Zeng W, Edwards AC, Duniho S, McEarchern JA, Law CL, Gerber HP, Grewal IS: **Blocking of CD27-CD70 pathway by anti-CD70 antibody ameliorates joint disease in murine collagen-induced arthritis.** *J Immunol* 2009, **183**:3770-3777.
 58. Manocha M, Rietdijk S, Laouar A, Liao G, Bhan A, Borst J, Terhorst C, Manjunath N: **Blocking CD27-CD70 costimulatory pathway suppresses experimental colitis.** *J Immunol* 2009, **183**:270-276.
 59. Kahan SM, Wherry EJ, Zajac AJ: **T cell exhaustion during persistent viral infections.** *Virology* 2015.
 60. De Milito A, Nilsson A, Titanji K, Thorstensson R, Reizenstein E, Narita M, Grutzmeier S, Sonnerborg A, Chiodi F: **Mechanisms of hypergammaglobulinemia and impaired antigen-specific humoral immunity in HIV-1 infection.** *Blood* 2004, **103**:2180-2186.
 61. Wolthers KC, Otto SA, Lens SM, Kolbach DN, van Lier RA, Miedema F, Meyaard L: **Increased expression of CD80, CD86 and CD70 on T cells from HIV-infected individuals upon activation in vitro: regulation by CD4+ T cells.** *Eur J Immunol* 1996, **26**:1700-1706.
 62. Matter M, Odermatt B, Yagita H, Nuoffer JM, Ochsenbein AF: **Elimination of chronic viral infection by blocking CD27 signaling.** *J Exp Med* 2006, **203**:2145-2155.
 63. Held-Feindt J, Mentlein R: **CD70/CD27 ligand, a member of the TNF family, is expressed in human brain tumors.** *Int J Cancer* 2002, **98**:352-356.
 64. Diegmann J, Junker K, Gerstmayer B, Bosio A, Hindermann W, Rosenhahn J, von Eggeling F: **Identification of CD70 as a diagnostic biomarker for clear cell renal cell carcinoma by gene expression profiling, real-time RT-PCR and immunohistochemistry.** *Eur J Cancer* 2005, **41**:1794-1801.
 65. Grewal IS: **CD70 as a therapeutic target in human malignancies.** *Expert Opin Ther Targets* 2008, **12**:341-351.
 66. Jacobs J, Deschoolmeester V, Zwaenepoel K, Rolfo C, Silence K, Rottey S, Lardon F, Smits E, Pauwels P: **CD70: An emerging target in cancer immunotherapy.** *Pharmacol Ther* 2015.

67. Schaer DA, Hirschhorn-Cymerman D, Wolchok JD: **Targeting tumor-necrosis factor receptor pathways for tumor immunotherapy.** *J Immunother Cancer* 2014, **2**:7.
68. Sanmamed MF, Pastor F, Rodriguez A, Perez-Gracia JL, Rodriguez-Ruiz ME, Jure-Kunkel M, Melero I: **Agonists of Co-stimulation in Cancer Immunotherapy Directed Against CD137, OX40, GITR, CD27, CD28, and ICOS.** *Semin Oncol* 2015, **42**:640-655.
69. Cormary C, Gonzalez R, Faye JC, Favre G, Tilkin-Mariame AF: **Induction of T-cell antitumor immunity and protection against tumor growth by secretion of soluble human CD70 molecules.** *Cancer Gene Ther* 2004, **11**:497-507.
70. Lorenz MG, Kantor JA, Schlom J, Hodge JW: **Anti-tumor immunity elicited by a recombinant vaccinia virus expressing CD70 (CD27L).** *Hum Gene Ther* 1999, **10**:1095-1103.
71. 2014. CT-hicrcRpo: **Celldex Therapeutics' Varlilumab Continues to Demonstrate Very Favorable Profile.** In *ASCO Annual Meeting 2014*. Edited by. Chicago, IL, USA; 2014.
- **72. Buchan SL, Manzo T, Flutter B, Rogel A, Edwards N, Zhang L, Sivakumaran S, Ghorashian S, Carpenter B, Bennett CL, et al.: **OX40- and CD27-mediated costimulation synergizes with anti-PD-L1 blockade by forcing exhausted CD8+ T cells to exit quiescence.** *J Immunol* 2015, **194**:125-133.
- 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	Effector CD8 ⁺ T cells	Memory CD8 ⁺ T cells
<p>Inducible CD70 expression (TLR4/9, IFNγ, CD40)</p> <p>DC</p> <p>MHC I TCR CD70 CD27 TRAFs</p> <p>Canonical and non-canonical NF-κB pathways</p> <p>CD8⁺ T cell</p> <p>↑ Anti-apoptotic molecules (BCL-XL) ↑ Cytokine receptors (IL-2Rα, IL-12β)</p>	<p>Augmented CD27 stimulation</p> <p>↑ Expansion of epitope-specific T cells</p> <p>Epitope-specific TCR</p> <p>Cytokines</p>	<p>Augmented CD27 stimulation</p> <p>↑ Number of epitope-specific T cells ↑ Secondary responses ● Retention of IL-7Rα expression</p> <p>IL-7Rα</p> <p>Renewal</p>
	<p>Diminished CD27 stimulation</p> <p>↓ Number of epitope-specific T cells ↓ Function ↓ Proliferation</p>	<p>Constitutive CD27 stimulation</p> <p>↓ Secondary responses ● Impaired protection</p>
		<p>Diminished CD27 stimulation</p> <p>↓ IL-7Rα expression ● Delayed recall ● Early reduction in virus-specific T cells</p>

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 <i>et al</i> , 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 <i>et al</i> , 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 <i>et al</i> , 2014 [Ref. 37]
		Tetramer-ICS & flow cytometry	~3-4 fold increase in IL-2 ⁺ , IFN γ ⁺ 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-7Ra expression on OT-1 CD8 ⁺ T-cells 3dpa, but ~2-fold 4dpa		
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 <i>et al</i> , 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 IFN γ ⁺ T-cells 10dpi	Constitutive CD27 co-stimulation during	
		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-		

	IAV then challenged 51-61 days later with a serologically distinct IAV	ICS and flow-cytometry	cells in the spleen and blood 8dpc ~4-fold decrease in the number of IFN γ ⁺ CD8 ⁺ T-cells 8dpc	both primary and secondary activation decreases recall	
	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	
CD27 ^{-/-}	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	Hendricks <i>et al</i> , 2003 [Ref. 12]
	Purified T-cells from WT and CD27 ^{-/-} mice were activated by α CD3 cross-linking +/- additional α CD28 <i>in vitro</i>	Thymidine incorporation	~2-fold decrease in proliferation 3dpa for T-cells from CD27 ^{-/-} mice in the absence of α CD28 α CD28 increases proliferation of CD8 ⁺ T-cells from CD27 ^{-/-} mice, but not to same extent as WT mice 3dpa	CD27 and CD28 co-stimulation are qualitatively different	
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 <i>et al</i> , 2013 [Ref. 39]
		Flow cytometry	Decreased differentiation by CD44 ^{hi} and CD62L expression 8dpi		
		ICS & flow-cytometry	~2-fold reduction in IFN γ , 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 IFN γ ⁺ TNF ⁺ cells 7dpi.	Loss of CD27 co-stimulation decreases the function of epitope-specific CD8 ⁺ T-cells	Penaloza-McMaster <i>et al</i> , 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 α CD70- 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-7R α expressing memory precursor cells	Dong <i>et al</i> , 2012 [Ref. 42]
		Flow-cytometry	~2-fold decrease in IL7R α 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 <i>et al</i> , 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 <i>et al</i> , 2001 [Ref. 48]
Human T-cells	CD3 ⁺ T-cells were activated by α CD3 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 <i>et al</i> , 2012 [Ref. 46]
		ICS	~2-fold increase in IFN γ and TNF production 2dpa		
Human T-cells	CD3 ⁺ T-cells were activated by α CD3 cross-linking with or without CD27 cross-linking with plate bound Varlilumab	ELISA	~4-fold increase in IFN γ , TNF, IL-2 and IL-13 production 72hpa	Increased CD27 co-stimulation enhances cytokine production	Ramakrishna <i>et al</i> , 2015 [Ref. 47]
	CD3 ⁺ T-cells cultures were activated with α CD3 cross-linking with irradiated CD70-expressing cells	ELISA	~2-fold increase in IFN γ and IL-13 production 72hpa		
	CD3 ⁺ T-cells were activated by α CD3 cross-linking with or without cross-linking CD27 with plate bound Varlilumab	Gene microarray	CD27 co-stimulation resulted in a distinct gene expression profile		

dpa=days post activation, hpa=hours post activation

Table 3. Publications investigating the influence of CD27 co-stimulation on $\gamma\delta$ T-cells

Model	Experimental design	Method of detection	Major findings	Key finding	Reference
Mouse studies					
C57BL/6	Total $\gamma\delta$ T-cells from spleen and LN were stimulated with α CD3 supplemented with sCD70	CBA and flow cytometry	Dose dependent increase in survival and expression of pro-inflammatory cytokines	CD27 co-stimulation supported survival and proliferation of $\gamma\delta$ T cells	Ribot <i>et al</i> , 2010 [Ref. 51]
WT and CD27^{-/-}	Mice were infected with murine herpes virus and malaria	ICS	~ 1 to 4 fold increase in proportion IFN γ producing $\gamma\delta$ T-cells	Loss of CD27 co-stimulation decreased IFN γ production	
C57BL/6	Thymic and splenic $\gamma\delta$ T-cells were isolated from embryonic, newborn and adult C57BL/6 mice	Flow cytometry	90% of $\gamma\delta$ thymocytes were CD27 ^{hi}	CD27 expression defines stable IFN γ -producing and IL-17-producing $\gamma\delta$ subsets	Ribot <i>et al</i> , 2009 [Ref. 1]
WT, TCR α- and TCRβ-deficient, CD27^{-/-}	Peripheral $\gamma\delta$ T-cells were isolated	Real time PCR and flow cytometry	Decrease in IFN γ expression levels in peripheral	Loss of CD27 co-stimulation decreases IFN γ expression	
FTOC $\gamma\delta$ thymocytes	FTOC $\gamma\delta$ thymocytes were treated with sCD70 and immunoglobulin	Real time PCR	Upregulation of IFN γ in CD27-expressing $\gamma\delta$ -thymocytes and down-regulation of IL-17 in CD27-negative $\gamma\delta$ -thymocytes	Enhanced CD27 co-stimulation affected IFN- γ and IL-17 expression	
Human studies					
γ9δ2 T-cell line	γ 9 δ 2 cells were enriched from phosphoantigen expanded PBMCs. MACS-sorted $\gamma\delta$ T-cells were stimulated with phosphoantigen in the presence of sCD70 or α CD70	CFSE and CBA	Augmented proliferation and increase in Th1 effector functions	Enhanced CD27 co-stimulation increases proliferation, survival and cytokine production	DeBarros <i>et al</i> , 2011 [Ref. 52]

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 α CD70 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 <i>et al</i> , 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 α CD70 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 <i>et al</i> , 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 α CD70 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 <i>et al</i> , 2009 [Ref. 58]
		Histology	~2-fold decrease in tissue destruction (histology score) 8wpa		
	Experimental colitis was established and mice were treated with or without α CD70 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 IL7R α expression	Constitutive CD27 co-stimulation deregulates differentiation	Van Gisbergen <i>et al</i> , 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 α ⁺) of CD8 ⁺ T-cells following restimulation		
C57BL/6	Mice were infected with chronic LCMV in the presence or absence of an α CD70 blocking mAb	ICS & flow-cytometry	~1.5-fold increase in the number of IFN γ 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 <i>et al</i> , 2011 [Ref. 40]
C57BL/6	Mice were infected with a chronic strain of LCMV in the presence or absence of an α CD70 blocking mAb	Immunological focus assay	~1.5-log decrease in viral titres 66dpi	Blocking CD27 co-stimulation increases viral clearance	Matter <i>et al</i> , 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. 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 <i>et al.</i> , 2015 [Ref. 72]
	Additional blockade of PD-1	ICS & flow-cytometry	~2-fold increase in the proportion of IFN γ ⁺ 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 <i>et al.</i> , 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 <i>et al.</i> , 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 α CD27 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, 1 patient partial response, 3 individuals stable disease		
dpi=davs post infection, dpa=davs post activation, dpt=davs post transfer, dpc=davs post challenge					

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