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CD27 Promotes CD4+ Effector T cell Survival in Response to Tissue Self-antigen

Kelly A. Remedios[1,2], **Lauren Meyer**[3], **Bahar Zirak**[1], **Mariela L. Pauli**[1], **Hong-An Truong**[2], **Devi Boda**[1], **Michael D. Rosenblum**[1]

[1]Department of Dermatology, University of California, San Francisco, CA 94143, USA

[2]TRex Bio, Burlingame, CA, 94010

[3]Department of Pediatrics, University of California, San Francisco, CA 94143, USA

Abstract

Signaling through CD27 plays a role in T cell activation and memory. However, it is currently unknown how this costimulatory receptor influences CD4$^+$ effector (Teff) cells in inflamed tissues. In the current study, we utilized a murine model of inducible self-antigen expression in the epidermis to elucidate the functional role of CD27 on auto-reactive Teff cells. Expression of CD27 on antigen-specific Teff cells resulted in enhanced skin inflammation when compared to CD27-deficient Teff cells. CD27 signaling promoted the accumulation of IFNg and IL-2-producing T cells in skin draining lymph nodes (SDLNs) in a cell-intrinsic fashion. Surprisingly, this costimulatory pathway had minimal effect on early T cell activation and proliferation. Instead, signaling through CD27 resulted in the progressive survival of Teff cells during the autoimmune response. Utilizing BH3 profiling to assess mitochondrial cell priming, we found that CD27-deficient cells were equally as sensitive as CD27-sufficient cells to mitochondrial outer membrane polarization upon exposure to either BH3 activator or sensitizer peptides. In contrast, CD27-deficient Teff cells expressed higher levels of active-caspase 8. Taken together, these results suggest that CD27 does not promote Teff cell survival by increasing expression of anti-apoptotic BCL2 family members but instead acts by preferentially suppressing the cell-extrinsic apoptosis pathway, highlighting a previously un-identified role for CD27 in augmenting autoreative Teff cell responses.

Introduction

Costimulatory receptors shape the initiation, magnitude and quality of an immune response. The prototypical costimulatory receptor is CD28 and stimulation through this receptor is required for optimal T cell activation and productive immunity (1). Additional costimulatory receptors include the TNFR super family (TNFRSF), which includes OX40, CD30, 4–1BB and CD27 (2). These TNFRSF family members lack pro-apoptotic death domains and signaling upon engagement with their respective ligands induces the activation of the NFkB and JNK pathways. While the functions of these receptors can be complementary, the

Address of Correspondence: Michael D. Rosenblum M.D., Ph.D., Medical Sciences Building, Health Sciences West - 1201B, 513 Parnassus Avenue, San Francisco, CA 94132, Ph: 415-476-1685, Michael.Rosenblum@ucsf.edu.

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outcome of signaling through any given receptor is highly context dependent. It is therefore of interest to understand how individual TNFRSF receptors influence immune responses within different tissues and inflammatory contexts.

One particular TNFRSF member, CD27, is constitutively expressed by naïve and memory T cells in secondary lymphoid organs as well as on activated B cells (3). The only known ligand for CD27 is CD70. Expression of CD70 is tightly regulated and only transiently expressed on dendritic cells (DCs), B cells, T cells and NK Cells after immune activation (4-6). Engagement of CD27 by CD70 induces the recruitment of the TRAF2 and TRAF5 adaptor proteins which in turn activate either the JNK or NFKb signaling pathways (7). This costimulatory pathway influences T cell function in several disease models (8). In settings of viral infection, CD27 contributes to the effective generation of both primary and memory CD8+ T cell responses (9-12). CD27 can also promote T cell responses in the context of productive immunization (13-16). Finally, constitutive expression of CD70 on DCs result in autoimmunity (17). The CD27 pathway is therefore a promising target for therapeutic intervention to either augment immune responses to infections and tumors or to attenuate excessive inflammation in the setting of autoimmunity.

Signaling through CD27 can augment T cell responses, in part by promoting cell survival. In specific contexts, engagement of CD27 can prevent apoptosis in both human and mouse CD8+ T cells by increasing expression of anti-apoptotic BCL2 family members (including BCL-XL) (18-20). CD27 can also promote survival by inducing the downregulation of FasL and suppressing extrinsic (or death-receptor mediated) apoptosis (21). In addition to regulating the expression of anti-apoptotic proteins, CD27 may also promote T cell survival and maintenance by modulating the expression of both cytokines and cytokine receptors. During viral infection, CD27 can induce IL-2 expression and promote CD8+ T cell survival through autocrine IL-2 signaling (22). CD27 signaling has also been implicated in increasing the frequency of IL-7 receptor expressing memory precursors (14). The mechanisms by which CD27 promotes T cell survival therefore appear to be highly complex and contextually dependent.

The molecular mechanisms responsible for CD27-mediated influence of T cell function have predominately been defined in CD8+ T cells. The role of CD27 signaling in CD4+ T cell responses remains poorly understood. In addition, how the CD27 pathway influences T cell responses outside of secondary lymphoid organs (*i.e.,* in peripheral tissues such as the skin) remains to be defined. In the current study, we use a well-established model of cutaneous autoimmunity to study the role of CD27 signaling in CD4+ Teff cell responses to tissue antigen (23).

Materials and Methods

Animals

All mice were bred and maintained in specific pathogen free facilities at UCSF according to NIH guidelines and experiments were approved by the Institutional Animal Care and Use Committee of UCSF. K5/TGO BALB/C (23) mice were backcrossed to C57BL/6 mice for at least 10 generations and crossed to CD45.1 mice purchased from Jackson Laboratories to

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generate K5/TGO/CD45.1.2 recipients. CD27−/− (9) mice were generously donated by Jannie Borst and crossed to OTII mice. Control donors were generated by crossing OTII to the CD45.1 strain.

Adoptive Transfer of T cells and Disease Development

Spleens and lymph nodes of donor WT OTII CD45.1 and CD27−/− OTII CD45.2 mice were harvested and processed. Single cell suspensions underwent TAC lysis to remove red blood cells followed by a magnetic CD4+ isolation (STEMCELL). Between 2×10^5 and 3×10^6 OTII cells (as noted in figure legends) were adoptively transferred i.v. into gender matched K5/TGO/CD45.1.2 recipients. Recipient mice were placed on a 1mg/kg doxycycline chow diet to turn on antigen in the skin. For clinical diseases measurement, dorsal hair was first shortened with clippers before applying depilatory cream (Nair) to the shaved region for a period of exactly 30 seconds before wiping clean.

Skin Processing for Flow Cytometry

Skin was processed and digested as previously described. Briefly, trunk skin was shaved, harvested, lightly defatted and minced. Skin was then incubated in digestion media: RPMI with 2mg/mL collagenase XI (Sigma, Catalog #C9407), 0.5mg/mL hyaluronidase (Sigma, Catalog #H3506) and 0.1 mg/mL DNase (ICN) for 45 minutes at 37C in a shaking incubator. Digested skin was then washed with RPMI, filtered, and then stained for flow cytometry.

Antibodies and Flow Cytometry:

Cells were stained for surface antigens and a live/dead marker (Ghost Dye™ Violet 510, Tonbo Biosciences) in PBS with 2% Calf Serum and 1% penicillin-streptomycin for 30 minutes at 4°C. To stain for intracellular cytokines, 4×10^6 cells were stimulated with Tonbo restimulation cocktail (Catalog #TNB-4975) in tissue culture media for 4 hours. Cells were then washed and surface antigens were stained in PBS with 2% FBS and 1% penicillin/streptomycin. Intracellular staining was performed using the eBioscience FOXP3 staining kit (Catalog #00–5523-00). Antibodies used are listed in Supplementary Table 1. Samples were run on a Fortessa (BD Biosciences) in the UCSF Flow Cytometry Core. FlowJo software (FlowJo, LLC) was used to analyze flow cytometry data.

BH3 Profiling

BH3 profiling was performed as previously described (24). Cells were isolated from the SDLNs of mice that received WT and CD27−/− OTII cells followed by CD4 enrichment via magnetic bead isolation (STEMCELL). Cells were then stained with with surface markers in 2% FBS and 1% penicillin/streptomycin. After the surface stain, cells were resuspended in MEB buffer at 2×10^6 cells/mL. Cells were then treated with various peptide concentrations in the presence of 0.001% digitonin and incubated for 1 hour at room temperature. After peptide exposure, cells were fixed in 4% PFA for 10 minutes, followed by neutralization with N2 buffer (1.7M Tris, 1.25M Glycine pH 9.1). Cells were then stained with anti-cytochrome C A647 (BD) in intracellular staining buffer (10%BSA, 2% Tween20 in PBS) at 4C overnight. Cells were analyzed by FACS the next day.

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BIM and PUMA peptides were dose titrated and used at final concentrations from 1uM-0.01uM and 10um- 0.1uM respectively. DMSO was used as a vehicle control (maximal mitochondrial cytochrome C retention) while 15uM alamethycin was used as a positive control by directly permeabilizing the mitochondria to induce full cytochrome C release. Percentage of cytochrome c release for each sample was calculated as % cytochrome c release = 1- (MFI_{sample} – MFI_{ALA}) / (MFI_{DMSO} – MFI_{ALA}).

Caspase 8 Staining

SDLNs were processed into a single cell suspension and stained for 30 minutes with the CaspGLOW Fluorescein Active Caspase-8 (Cat# 88–7005) staining kit and protocol. Following active caspase staining, cells were stained with surface markers and analyzed by FACS.

Statistics

Statistics were performed using GraphPad Prism softward. P-values were calculated using two-tailed paired or unpaired students t-tests.

Results

CD27 expression by antigen-specific CD4+ Teff cells promotes skin inflammation

To study the role of CD27 in CD4+ T cell-mediated autoimmunity, we utilized a model of inducible antigen expression within the epidermis (23, 25, 26). This model involves two transgenes, one which has a cytokeratin 5 (K5) promoter driving the expression of a tetracycline trans-activator protein and the other transgene drives the expression of the model antigen ovalbumin (OVA) under the control of a tetracycline-response element (TGO). Mice with both K5 and TGO transgenes enable expression of OVA to be stringently induced in the basal layer of the epidermis upon treatment with doxycycline. The transfer of ovalbumin-specific CD4+ T cells (OTII) into K5/TGO recipients treated with doxycycline induces a robust inflammatory dermatitis.

We used the K5/TGO model to elucidate the role of the CD27 pathway on autoreactive CD4+ Teff cells specific for a tissue antigen. CD27–/– mice were crossed to OTII-TCR transgenic mice, and resultant mice were used as a source of ova-specific T cells that lacked CD27 expression. CD4+ T cells were isolated from either WT OTII or CD27–/– OTII mice and transferred into K5/TGO recipients. Mice were then treated with doxycycline to induce antigen expression in the epidermis. Recipient mice that received WT OTII cells developed a robust inflammatory dermatitis that progressively worsened over the course of 11 days (Figure 1A-B). This dermatitis was accompanied by an increase in percentage and absolute numbers of neutrophils (CD45+CD11b+Ly6G+) and inflammatory macrophages (CD45+CD4-CD8-MHCII+CD11b+Ly6Chi) in skin (Figure 1C-D). In contrast, mice receiving CD27–/– OTII cells had attenuated skin disease, reduced scaling, and decreased neutrophil and inflammatory macrophage influx in skin. These results demonstrate that CD27 expression on antigen-specific CD4+ Teff cells promotes autoimmunity to tissue antigen.

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CD27 promotes the accumulation of IFNγ- and IL-2-producing autoreactive CD4+ Teff cells.

We next evaluated how CD27 expression influenced the accumulation of antigen-specific CD4+ T cells in skin and SDLN upon antigen induction. WT OTII or CD27−/− OTII cells were adoptively transferred into K5/TGO recipients and mice were treated with doxycycline. After 9 days of antigen induction, skin and SDLNs were harvested and the accumulation of antigen-specific T cells was quantified. In skin, CD27−/− OTII cells accumulated at lower percentages and absolute numbers compared to WT OTII cells (Figure 2A) and a similar trend was seen in SDLNs, although this was not statistically significant (Figure 2B). We have previously shown that in this model, ova-specific T cells almost exclusively express IFNg (25). We therefore quantified expression of this cytokine *via* intracellular cytokine staining by flow cytometry. When compared to WT OTII cells, CD27−/− OTII cells produced significantly less IFNg and IL-2 (Figure 2C-D). Taken together, these results suggest that CD27 expression promotes the accumulation of cytokine-producing autoreactive CD4+ Teff cells in response to autoantigen expression in skin.

CD27 promotes autoreactive Teff cell accumulation in a cell-intrinsic manner.

To determine if CD27 signaling promotes autoreactive CD4+ T cell accumulation in a cell-intrinsic or cell-extrinsic fashion, WT OTII and CD27−/− OTII cells were combined at a 1:1 ratio and adoptively transferred into K5/TGO recipients. Accumulation of WT and CD27−/− OTII cells was quantified in SDLNs at days 3, 5, 7 and 10 after antigen induction (Figure 3). On days 3 and 5 after antigen induction, both WT and CD27−/− OTII cells were present at equivalent percentages, suggesting that CD27 does not markedly influence the early CD4+ T cell response to tissue antigen. However, by day 7, WT OTII cells were present at approximately 20-fold higher levels compared to CD27−/− OTII cells and this difference was maintained at day 10. These results suggest that CD27 signaling promotes autoreactive Teff cell accumulation in a cell-intrinsic fashion and this effect is most pronounced approximately 7–10 days after antigen presentation.

CD27 promotes autoreactive Teff cell survival but not proliferation.

To determine the mechanism by which CD27 promotes the accumulation of antigen-specific CD4+ T cells in our adoptive transfer model, we first quantified proliferation. WT OTII CD45.1 or CD27−/− OTII CD45.2 cells were CFSE labeled, combined at a 1:1 ratio and co-transferred into K5/TGO recipients. Recipients were then placed on doxycycline to induce antigen expression. SDLNs were harvested 4 days after transfer and CFSE dilution quantified by flow cytometry. WT and CD27−/− OTII cells displayed similar levels of proliferation with no differences was observed in either the proliferative or division index of each population (Figure 4A). We also quantified Ki67 expression as a metric of cell cycling. Both WT and CD27−/− cells expressed similar levels of Ki67 (Figure 4B). Consistent with these results, both WT and CD27−/− cells expressed similar levels of CD69, a validated marker of early T cell activation (Figure 4C). These results suggest that signaling through CD27 does not promote Teff cell accumulation by augmenting early T cell activation, entry into the cell cycle or cumulative cell proliferation.

We next assayed whether signaling through CD27 affected Teff cell survival in our model. Similar to above, both WT OTII CD45.1 and CD27−/− OTII CD45.2 cells were adoptively

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transferred at a 1:1 ratio into K5/TGO recipients followed by antigen induction. Survival was assessed at days 5 and 7 after transfer by quantifying the percentage of dead and dying cells, using Ghost dye (an amine reactive viability dye) and Annexin V staining (Figure 4D). A significant increase in dead or dying Teff cells (GHOST+Annexin V+) was observed in the CD27−/− OTII population compared to WT, and this trend increased with time after antigen induction (Figure 4D). Taken together, these results suggest that CD27 augments the accumulation of autoreactive CD4+ T cell responding to tissue antigen by promoting cell survival rather than activation or proliferation.

Signaling through CD27 suppresses the extrinsic cell death pathway to promote autoreactive Teff cell survival.

One mechanism by which multiple TNFRSF receptors promote survival is through the induction of anti-apoptotic BCL2 family members (20, 27-30). These anti-apoptotic proteins suppress cell death by antagonizing both multidomain pro-apoptotic proteins (BAX and BAK) as well as activator BH3-only proteins (BID and BIM). Is this manner, anti-apoptotic BCL2 family members can prevent mitochondrial outer membrane permeabilization (MOMP) and the release of cytochrome *c* (31). Opposing anti-apoptotic proteins are sensitizer BH3-only proteins. This group of BH3 family members bind to anti-apoptotic proteins, inhibiting their function and allowing for the release of activators and eventual activation of BAX and BAK. This process is referred to as the mitochondrial or intrinsic apoptotic pathway, and whether a cell undergoes apoptosis is ultimately regulated by the balance of pro-apoptotic, anti- apoptotic, and sensitizer BH3-only family members (reviewed in (31)). CD27 has been reported to induce the expression of anti-apoptotic proteins including BCL-XL and MCL-1(18-20). Thus, we hypothesized that signaling through CD27 promotes Teff cell survival by suppressing MOMP through the induction of anti-apoptotic proteins. To test this hypothesis, we utilized BH3 profiling. BH3 profiling is a powerful technique that can be utilized to assess mitochondrial priming (24). This technique involves exposing cells to peptides that mimic the BH3 domains of endogenous activator or sensitizer BH3-only proteins and then measuring the degree of cytochrome c release from mitochondria. Peptide mimics of global sensitizers BIM and PUMA, will inhibit all members of the anti-apoptotic family. BIM peptide mimics will also directly interact with BAX and BAK, whereas PUMA cannot. This technique can therefore be used to compare differential sensitivity of individual populations to mitochondrial apoptosis by measuring cytochrome c release in response to various BH3-only peptides (32). If a certain population of cells is more primed for mitochondrial apoptosis, such a population should release more cytochrome c in response to exposure to an activator BH3 peptide such as BIM. Differences in response to treatment with PUMA further illustrate that that a population exhibits a dependence on anti-apoptotic family members for survival.

We performed BH3 profiling on WT and CD27−/− OTII cells isolated from the SDLNs of recipient K5/TGO mice 4 days after antigen induction. Isolated cells were exposed to various concentrations of peptides or vehicle controls, then fixed and stained for mitochondrial cytochrome c. Assay validation confirmed that both BIM and PUMA peptide exposure induced a dose-dependent release of mitochondrial cytochrome c (Figure 5). Strikingly, in contrast to our hypothesis, both BIM and PUMA induced equal cytochrome c

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release in both WT and CD27−/− OTII cells at all concentrations tested, suggesting that these populations are equally primed for MOMP (Figure 5). Thus, differences in survival between autoreactive WT and CD27−/− OTII cells in our model is most likely not attributed to activity of anti-apoptotic BCL2 family members.

The other major mechanism that influences survival is the extrinsic, or death receptor mediated apoptosis pathway. The ligation of death receptors, such as TNFR, CD95, or TRAIL1 can induce the activation of caspase-8 *via* proteolytic cleavage (reviewed in (33)). Caspase-8 activation leads to the downstream caspase cascade that ultimately results in apoptosis. To determine if CD27 expression suppressed the extrinsic apoptosis pathway, we quantified active-caspase 8 by flow cytometry. At day 4 post-antigen induction, CD27−/− OTII cells expressed significantly higher levels of active caspase 8 compared to WT controls (Figure 5C). These results suggest that CD27 promotes Teff cell survival by suppressing the extrinsic apoptosis pathway.

Discussion

Developing therapeutics that target the immune system are of great interest to treat both autoimmunity and cancer. The latest generation of cancer immunotherapies targeting immunoregulatory receptors on T cells (*i.e.,* checkpoint inhibitor therapy) have had enhanced response rates in many tumors compared to conventional therapies. Notably, when checkpoint inhibitors are used in combination, response rates are higher than either therapy alone (34). In addition to “removing the brakes” from the immune response, it is also of great interest to understand how to “add fuel to the fire” and augment T cell responses. One way to achieve this is through targeting costimulatory receptors, including those of the TNFRSF (35). TNFRSF receptors play crucial roles in augmenting T cell responses by promoting activation, expansion, survival, differentiation and memory. It is therefore of fundamental importance to understand how these receptors function in different disease settings and within different tissues. In current study, we demonstrate that one TNFRSF member, CD27, enhances cutaneous immune responses by promoting autoreactive CD4+ Teff cell survival by suppressing the extrinsic (death receptor-mediated) apoptosis pathway.

Our findings are consistent with prior studies demonstrating that CD27 plays a major role in promoting T cell survival (14, 18-20, 22). Several studies have demonstrated that this pathway increases the expression of anti-apoptotic BCL-2 family members in CD4 and CD8 T cells, including BCL-2 and BCL-XL (18-20). We utilized BH3 profiling to test whether CD27 signaling suppressed sensitivity to mitochondrial induced cell death by increasing the expression of anti-apoptotic BCL2 proteins in autoreactive CD4+ Teff cells responding to tissue self-antigen (24). If increasing the expression of anti-apoptotic proteins was the major mechanism by which CD27 promoted Teff survival, CD27-deficient cells would be more sensitive to cytochrome c release upon treatment with either BIM (activator) or PUMA (sensitizer) peptides. Notably, we did not observe any difference in priming between CD27-sufficient or CD27-deficient cells. While our study does not necessarily rule out a role for CD27 in promoting survival through increasing the expression of anti-apoptotic proteins, our results strongly suggests that this is not the major mechanism in CD4+ Teff cells. This is consistent with previous work demonstrating that forced over-expression of BCL2 family

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members in CD8+ T cells alone or in combination was not enough to compensate for CD27-deficiency (19), suggesting that CD27 can promote survival through multiple mechanisms.

We found that CD27 expression on CD4+ Teff cells promotes survival *via* suppressing the extrinsic apoptosis pathway. Death-receptor ligation results activation of caspase-8 which in turn initiates the downstream caspase cascade which ultimately leads to apoptosis. CD27-sufficent cells expressed lower levels of active-caspase 8 compared to CD27-deficient cells. This finding, combined with our results demonstrating that antigen-specific Teff cells are equally sensitive to BH3-peptide induced MOMP with and without CD27, strongly suggests that CD27 promotes survival by suppressing activation of the extrinsic apoptosis pathway.

Our results are consistent with other models of tissue autoimmunity, demonstrating that blocking CD27 suppresses experimental colitis and lung allergy (36, 37). In contrast to these studies, it has been shown that CD27 deficiency augments autoimmune disease in experimental autoimmune encephalomyelitis (EAE) (38). Although these studies appear to be contradictory, CD27 is now known to play different roles in CD4+ T cell function depending on the inflammatory context. Multiple studies have demonstrated that while CD27 augment Th1 responses(15, 18, 39-41), signaling through this pathway attenuates Th17 responses (38, 41). The K5/TGO model used in this study induces a strong Th1 response, as defined by IFNg and IL-2 production from the transferred antigen specific Teff cells, with minimal IL-17 production (25). We observed a decrease in IFNg and IL-2 production from CD27-deficient cells after antigen induction, with no difference in IL-17 (data not shown). Thus, our results support the current literature, demonstrating that CD27 enhances Th1 responses. The functional consequence of CD27 signaling in Th17-driven cutaneous inflammatory responses (such as psoriasiform inflammation or *c. albicans* infection) remain to be determined. Interestingly, alterations in the CD70-CD27 pathway have been associated with disease severity in patients with systemic lupus erythematosus (SLE), a predominantly Th1-mediated disease with numerous skin manifestations (42). Thus, the mouse model utilized in our study and our major findings may have relevance to human SLE.

In conclusion, in this study we demonstrate that CD27 plays a critical role in augmenting cutaneous autoimmunity by promoting autoreactive CD4+ T cell survival. These results suggest that manipulating the CD27 pathway may be a promising therapeutic approach in autoimmune or malignant diseases of the skin.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Key Points:

- The CD27 pathway on $CD4^+$ T cells plays a role in driving skin autoimmunity
- CD27 signaling promotes survival by suppressing cell-extrinsic apoptosis

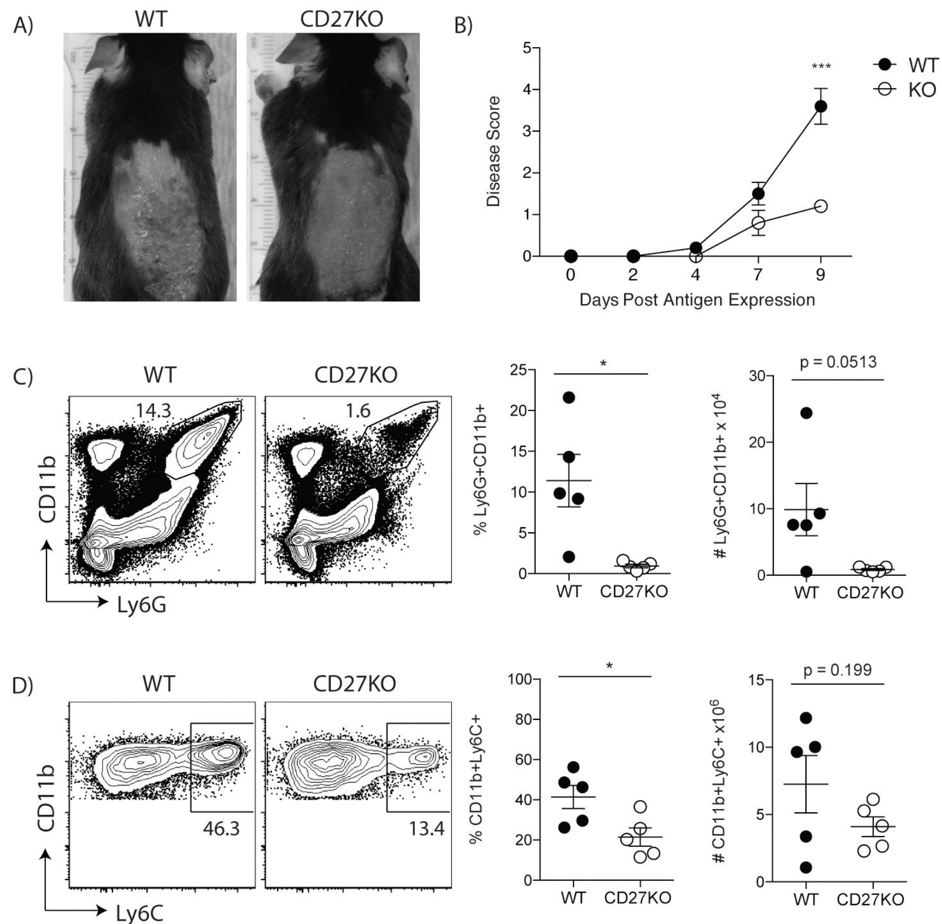

Figure 1: CD27 expression on CD4+ T cells promotes inflammation in response to tissue self-antigen.

WT OTII or CD27−/− OTII cells were adoptively transferred i.v. into K5/TGO recipients. The day of transfer, mice were placed on doxycycline chow to induce antigen expression. (A) Representative photos of dorsal skin of mice given WT or CD27−/− cells 9 days after antigen induction. (B) Clinical disease scores after antigen induction. (C) Representative flow cytometric plots and quantification of neutrophil (gated on CD45+CD11b+Ly6G+) percentages and absolute numbers in skin. (D) Representative flow cytometric plots and quantification of (E) percentages and (F) absolute number of pro-inflammatory (Ly6C+) and non-inflammatory (Ly6C-) macrophages (gated on Live CD45+ CD4-CD8-CD11b +MHCIIhi) in skin. Data is representative of 2 independent experiments with n=5 mice per group. P-values are determined using an unpaired students t-test. *$p<0.05$, *** $p<0.001$, ns = $p>0.05$.

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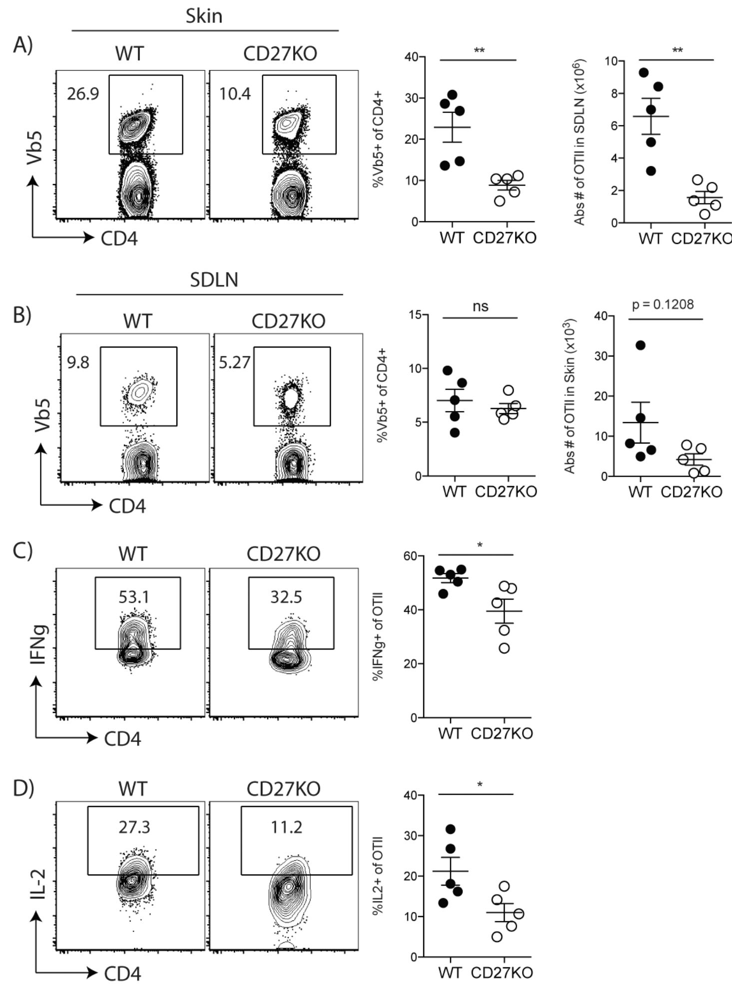

Figure 2: CD27 promotes accumulation of cytokine-producing antigen-specific CD4+ T cells in response to tissue self-antigen.

WT OTII CD45.1 or CD27−/− OTII CD45.2 cells were adoptively transferred i.v. into K5/TGO CD45.1.2 recipients. The day of transfer, mice were placed on doxycycline chow to induce antigen expression. Skin and SDLN were harvested at day 9 to quantify the accumulation and cytokine production from adoptively transferred T cells. (A-B) Representative flow cytometric plots (gated on Live CD4+ Vb5+ cells) and quantification of percentages and absolute numbers of transferred Vb5+ cells in the (A) SDLN and (B) skin. (C-D) Representative flow cytometric plots and quantification of (C) IFNg and (D) IL-2 producing OTII cells in the SDLN. Data is representative of 3 independent experiments with n=5 mice per group. P-values are determined using an unpaired students t-test. *p<0.05, *** p<0.001, ns = p>0.05.

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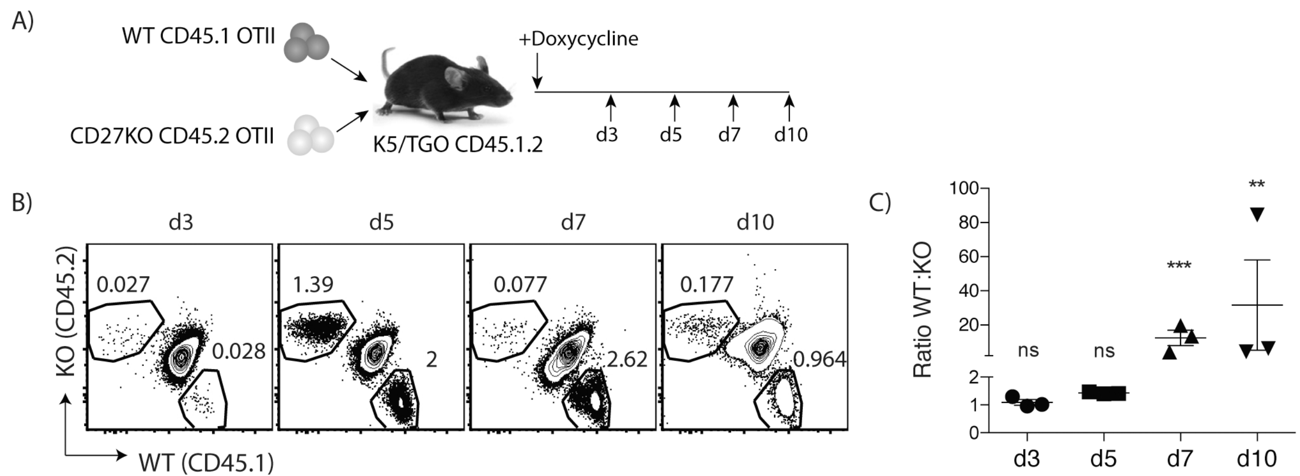

Figure 3: CD27 promotes CD4+ T cell accumulation in a cell-intrinsic manner.

(A) Experimental model. WT OTII CD45.1 and CD27−/− OTII CD45.2 cells were combined at a 1:1 ratio and cells were adoptively transferred into K5/TGO CD45.1.2 recipients and placed on doxycycline chow. (B) Representative flow cytometric plots of the percentages of WT and CD27−/− OTII cells at specific timepoints after antigen induction (gated on Live CD4+). (C) Quantification and ratios of WT:CD27−/− cells in the SDLN at days 3, 5, 7 and 10 after antigen induction. Statistics calculated using an unpaired students T test to compare percentage of WT vs CD27−/− cells at each timepoint. Data is representative of 2 independent experiments with 3 mice per timepoint. Graphs depict mean ± SD and p-values are determined using an unpaired students t-test. **$p<0.01$, ***$p<0.001$, ns = $p>0.05$.

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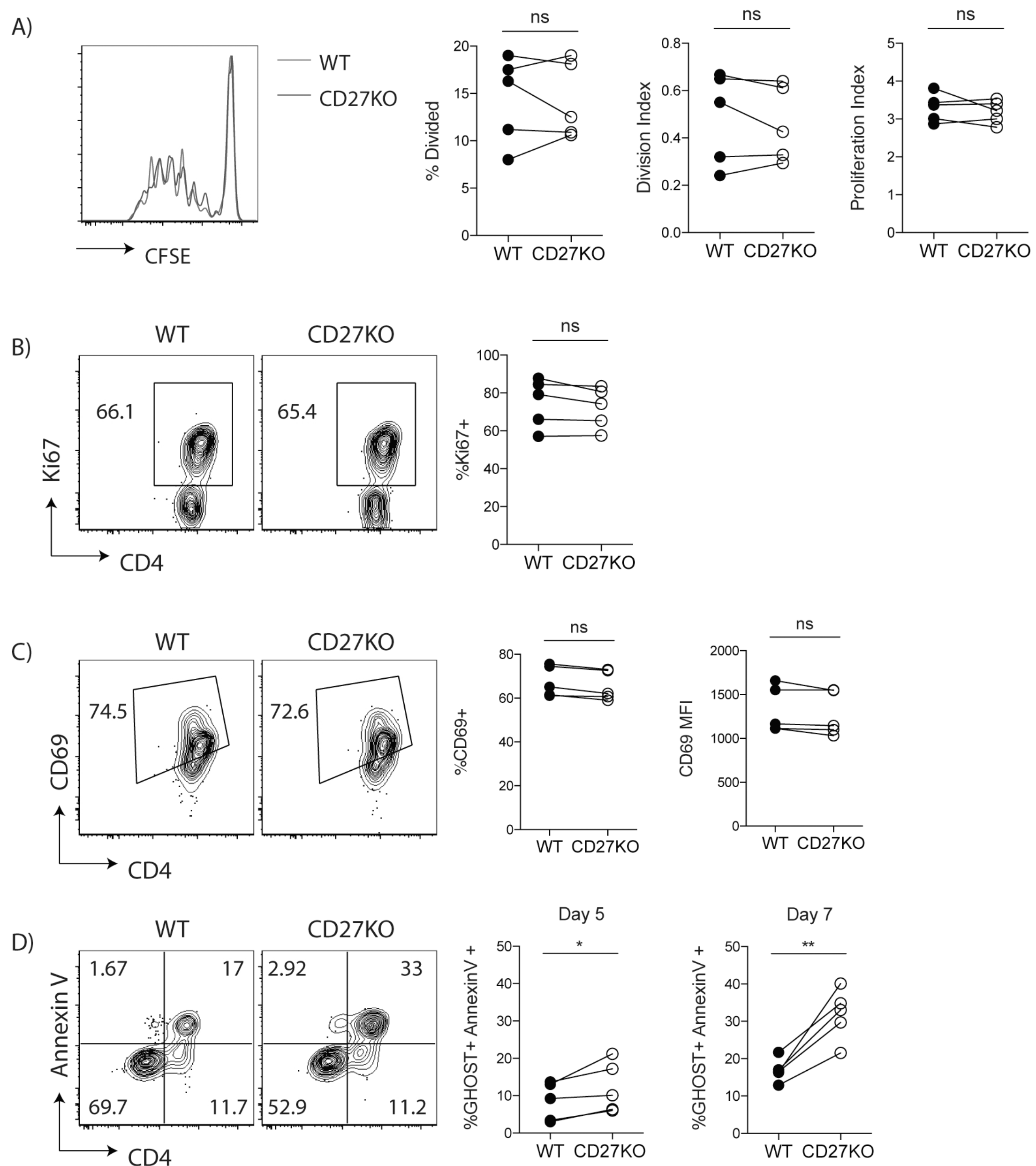

Figure 4: CD27 promotes CD4+ T cell survival in response to tissue self-antigen.

(A) WT OTII CD45.1 and CD27−/− OTII CD45.2 cells were CFSE labeled, combined at a 1:1 ratio and adoptively transferred into K5/TGO CD45.1.2 recipients placed on doxycycline chow to induce antigen expression. 4 days after transfer, SDLN were harvested and CFSE dilution of the transferred WT and CD27−/− populations (gated on Live CD4+ then CD45.1 or CD45.2 respectively) was quantified. (B) Representative flow cytometric plots and quantification of percentages of Ki67+ WT or CD27−/− OTII cells 4 days after transfer. (C) Representative flow cytometric plots and quantification of percentages and MFI of CD69 in WT or CD27−/− OTII cells 4 days after transfer. (D) Representative flow cytometric plots and quantification of cell death in WT or CD27−/− OTII cells as assessed by Annexin V and viability dye incorporation. Data is representative of at least two independent experiments

with n=5 or more mice. P-values are determined using a paired students t-test. *p<0.05, **p<0.01, ns = p>0.05.

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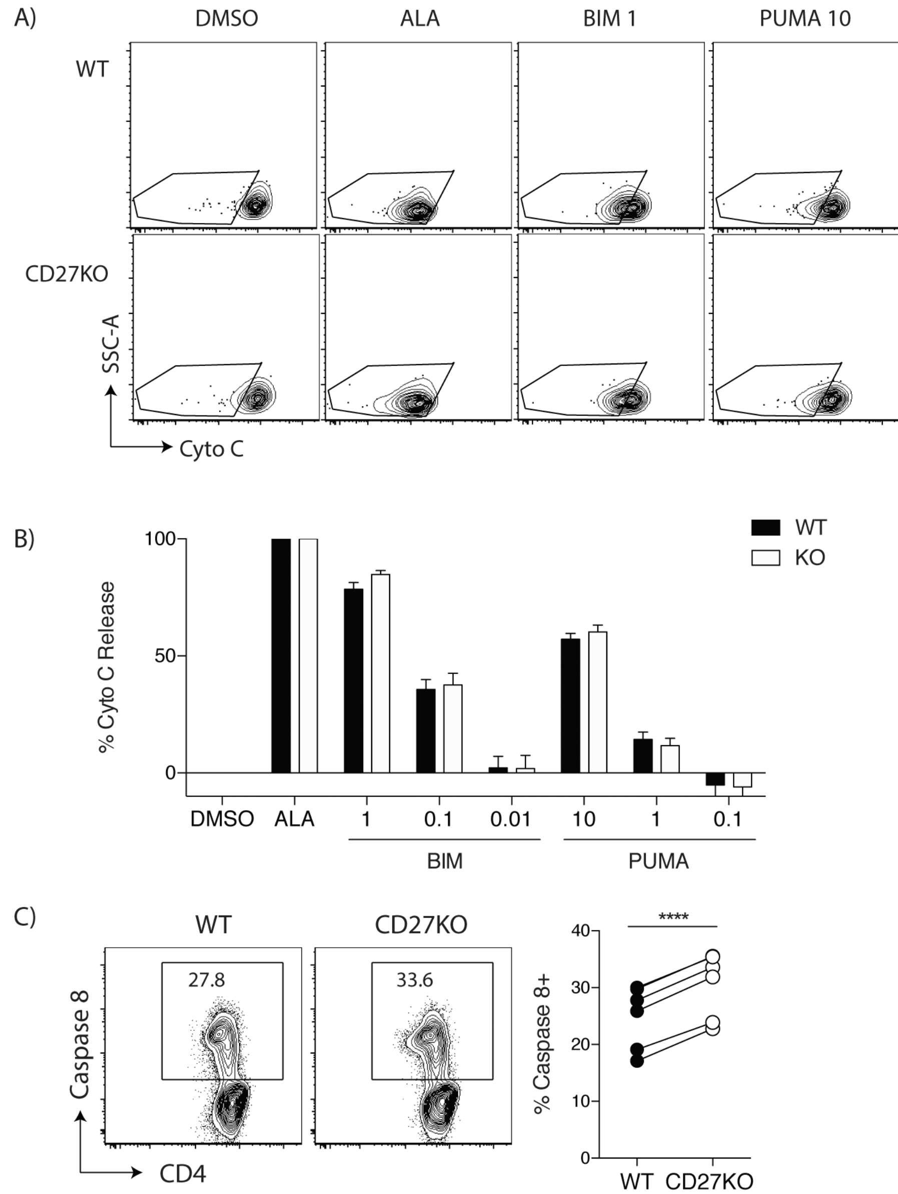

Figure 5: CD27 signaling on CD4+ T cells preferentially promotes survival *via* the extrinsic apoptosis pathway.

WT CD45.1 and CD27−/− CD45.2 OTII cells were combined at a 1:1 ratio and adoptively transferred into K5/TGO CD45.1.2 recipients followed by doxycycline administration. 4 days after antigen induction, SDLNs were harvested. After CD4 enrichment, BH3 profiling was performed using specific concentrations (as indicated in figure) of BIM (activator) peptide, PUMA(sensitizer) peptide, DMSO (vehicle), or ALA (positive control). Cytochrome C release was quantified by flow cytometry. (A) Representative flow cytometric plots of cytochrome C staining in either WT (top) or CD27−/− (bottom) cells, gated on Live CD4+ then either CD45.1 or CD45.2 respectively. (B) Quantification of percentage cytochrome c release after various peptide or vehicle treatments. (C) Flow cytometric staining and quantification of active caspase 8 expression in either WT (CD45.1+) or

CD27−/− (CD45.2+) cells, after gating of Live CD4+Vb5+. Data is representative of 3 independent experiments with n=4 mice. Graphs depict mean ± SEM. ****p<0.0001.

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