Molecular mechanisms underlying CD27-CD70 costimulation
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Chapter 6

CD27 instructs CD4⁺ T cells to provide help for the memory CD8⁺ T cell response after protein immunization

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CD27 Instructs CD4+ T Cells to Provide Help for the Memory CD8+ T Cell Response after Protein Immunization

Yanling Xiao, Victor Peperzak, Anna M. Keller, and Jannie Borst

For optimal quality, memory CD8+ T cells require CD4+ T cell help. We have examined whether CD4+ T cells require CD27 to deliver this help, in a model of intranasal OVA protein immunization. CD27 deficiency reduced the capacity of CD4+ T cells to support Ag-specific CD8+ T cell accumulation at the tissue site after primary and secondary immunization. CD27-dependent CD4+ T cell help for the memory CD8+ T cell response was delivered during priming. It did not detectably affect formation of CD8+ memory T cells, but promoted their secondary expansion. CD27 improved survival of primed CD4+ T cells, but its contribution to the memory CD8+ T cell response relied on altered CD4+ T cell quality rather than quantity. CD27 induced a Th1-diagnostic gene expression profile in CD4+ T cells, which included the membrane molecule MS4A4B. Accordingly, CD27 increased the frequency of IFN-γ- and IL-2-producing CD4+ T cells. It did not affect CD40L expression. Strikingly, MS4A4B was also identified as a unique marker of CD8+ memory T cells that had received CD27-proficient CD4+ T cell help during the primary response. This apparent iniminating effect suggests a role for MS4A4B as a downstream effector in CD27-dependent help for CD8+ T cell memory. The Journal of Immunology, 2008, 181: 1071–1082.

To generate optimal immunity toward the target pathogen, infection or vaccination should elicit expansion of Ag-specific T cells, their differentiation into effector T cells, and their accumulation at the site of antigenic challenge. Whereas TCR stimulation is a prerequisite for naive T cells to participate in the response, signals provided by costimulatory and cytokine receptors modulate size and quality of the responder populations (1–4). Such effects may be exerted during priming, but also throughout the T cell response, at different tissue locations and cell-to-cell interfaces.

Upon maturation, dendritic cells (DC) become the optimal elements for T cell priming. This involves Ag presentation, cytokine secretion, and cell surface expression of costimulatory ligands (5). CD80 and CD86, the ligands for CD28, are well recognized as important attributes of mature DC. Appreciation is now increasing for CD70, the ligand of costimulatory receptor CD27, as an important contributor to T cell priming at the T cell/DC interface. CD70 can be expressed by activated T and B cells and by all conventional DC subsets upon their maturation (6–9). CD70 expression on both CD8+ and CD11b+ DC is optimal after combined stimulation of TLR and CD40 (9), which reflects pathogen recognition and interaction with cognate CD4+ T cells, via CD40L. In such a scenario, DC are functionally modified (“licensed”) to support the CD8+ T cell response (10–13).

Ballock and Yagita showed that the capacity of CD40-stimulated, peptide-loaded DC to elicit a CD4+ T cell-independent primary CD8+ T cell response was fully dependent on the collective functions of CD70 and CD80/CD86 (14). Using blocking Ab in vivo, other authors have corroborated the importance of CD70 on DC for CD8+ T cell priming using protein immunization and infection models (9, 15, 16). These findings suggest that induction of CD70 expression is an important aspect of DC licensing by CD4+ T cells.

Under noninflammatory conditions, CD4+ T cells may deliver help to the primary CD8+ T cell response. This is the case, for instance, upon immunization with intact protein that contains both MHC class I- and class II-restricted epitopes and relies on cross-presentation by DC (10). The primary CD8+ T cell response to many infectious agents is CD4+ T cell independent, however, because TLR stimuli and inflammatory signals offered by infectious agents directly activate APC. The memory CD8+ T cell response, in contrast, is CD4+ T cell dependent, regardless of whether priming conditions are inflammatory or not (10). CD4+ T cell help delivered during the primary response generates memory CD8+ T cells of optimal quality; that is, they are long-lived and can respond to renewed antigenic challenge by efficient secondary expansion and exertion of effector functions (10, 17–20). The contribution of CD4+ T cells to this apparent memory CD8+ T cell programming is relevant for vaccination strategies and is the subject of intense scrutiny (10).

In agreement with the abovementioned effects of CD70, CD27 was found to complement CD28 in promoting the primary CD8+ T cell response in influenza virus infection and allograft models (21, 22). CD27 protects primed T cells from apoptosis and thus increases the size of the effector CD8+ T cell pool at priming and tissue sites (21). Effects of CD27 on effector CD8+ T cell accumulation are particularly apparent in the memory response (23, 24). CD27/CD70 interactions counteract contraction of the CD8+ effector T cell pool and thereby promote memory T cell formation (24, 25). Additionally, CD8+ T cells require CD27 for optimal secondary expansion (24).
The relevance of CD27/CD70 interactions for the CD4+ T cell response is less well examined. It is clear from studies in CD27−/− and CD70 transgenic mice that CD27/CD70 interactions can promote CD4+ effector T cell accumulation, most evidently in the lung after influenza virus infection (23, 26). In the same virus infection model, as well as after protein immunization, CD27 deficiency did not affect the B cell response (27). These findings suggest a role for CD27 on CD4+ T cells in CD8+ T cell rather than B cell help. Recently, CD70 on DC was found to initiate a pathway for Th1-type CD4+ effector T cell differentiation (28), which is in line with improved IFN-γ production in CD4+ T cells of CD70 transgenic mice (26). We have recently found that upon de novo synthesis in maturing DC, CD70 is routed to MHCI class II compartments, resulting in a simultaneous recruitment of CD70 and MHCI class II to the immunological synapse upon cognate contact with CD4+ T cells (29). These findings emphasize the importance of CD27/CD70 costimulation at the interface between CD4+ T cells and DC.

We have examined whether CD27 contributes to the capacity of CD4+ T cells to provide help to the CD8+ T cell response using a protein cross-presentation model. It was found that CD27 instills specific functional activities into primed CD4+ T cells, which are part of a Th1-type differentiation profile. The functional impact of CD27 on CD4+ T cells is important for their CD8+ T cell helper activity, in particular to endow memory CD8+ T cells with the potential to accumulate after secondary challenge. We have identified a molecular imprint of CD27-proficient CD4+ T cell help in CD8+ memory T cells in the form of the MS4A4B molecule.

Materials and Methods

Mice

Wild-type (WT), CD27−/− (23), OT-II, and CD27−/−OT-II transgenic mice on a C57BL/6 background were used for experiments at 6–12 wk of age. Experiments were approved by the Experimental Animal Committee of The Netherlands Cancer Institute and performed in accordance with national and institutional guidelines. OT-II mice express a transgenic TCR with specificity for OVA323-339 peptide in the context of H-2I-Ab (30). Mice were genotyped by PCR, and phenotypes were confirmed by flow cytometry. Mice were of the CD45.2 allotype unless specified otherwise.

Intranasal immunization and adoptive transfer

OVA protein and cholera toxin (CT) were purchased from Sigma-Aldrich and stored in aliquots at −20°C. Mice were anesthetized by methoxyflurane inhalation and immunized intranasally with 500 μg OVA protein plus 1 μg CT in 50 μl HBSS. Rechallenge with OVA protein or 50 μg OVA257-264 peptide was done in the same way. CT was used to enhance tissue uptake of protein via the mucosa (31). For adoptive transfer, purified splenic OT-II transgenic T cells were injected i.v. into recipient mice at 5 × 106 per mouse, or in the number indicated, suspended in 200 μl HBSS with 10% mouse serum. Mice were immunized at 2 days and 6 wk after adoptive transfer for primary and memory responses.

Preparation of purified T cells

For T cell purification from OT-II mice, nonadherent splenocytes were incubated on ice for 30 min with mAb RA3-6B2 to B220 and M5/114.15.2 to MHC class II, followed by 30 min incubation on ice with 100 μl goat anti-mouse Ig-coated magnetic beads and 20 μl sheep anti-rat Ig-coated magnetic beads (Advanced Magnetics) per 106 cells. Beads were removed by magnetic sorting. Purity of the resulting T cell populations was checked by flow cytometry using anti-TCR Vβ1/5.2 and anti-CD4 mAbs and was always >90%.

Flow cytometry

Lungs, spleens, and lung-draining lymph nodes (DLN) were forced through a nylon mesh in IMDM with 4% FCS to acquire single-cell suspensions. Erythrocytes were lysed on ice for 1 min in 0.14 M NH4Cl, 0.017 M Tris-HCl (pH 7.2). Next, cells were incubated with specific Abs conjugated to FITC, PE, or allophycocyanin. Allophycocyanin-labeled tetramers of murine MHCI class I H-2Kβ, β2-microglobulin, and OVA257-264 peptide (sequence: SIINFEKL) were prepared as described and used in combination with anti-CD8 mAb (32). Cells were analyzed using a FACSCalibur (BD Biosciences) and FCS Express software (De Novo Software). Propidium iodide-stained dead cells were excluded from analysis. mAbs used for immunofluorescence were anti-CD3, 500A2; anti-CD4, RM4-5; anti-CD8β, 53-5.8; anti-TCR Vβ5.1/5.2, MR9-4; anti-CD27, LG3A10; anti-CD45R/B220, RA3-6B2; anti-CD45.1, A20; anti-CD45.2,
CD27 controls CD4+ T cell help for the CD8+ T cell response

104; anti-IL-2, JES6-5H4; anti-IFN-γ, H9253; XMG1.2; anti-CD40L, MR1; and anti-CD25 (IL-2 receptor chain), PC-61. All of these Abs were obtained from BD Biosciences or were prepared as purified Ig from available hybridomas. mAb to granzyme B, PeliCluster GB-11, was from Sanquin.

Intracellular staining

To determine IL-2, IFN-γ, and CD40L production by CD4+ T cells directly ex vivo, WT and CD27−/− OT-II transgenic mice were immunized intranasally with OVA protein plus CT. At indicated time points after immunization, single-cell suspensions from spleen were prepared and stimulated with the murine MHC class II I-Aβ binding peptide OVA323–339 (sequence: ISQAVHAAHAEINEAGA, 100 μg/ml) for 5 h at 37°C and 5% CO2 in the presence of recombinant human IL-2 (40 U/ml, Chiron) and GolgiPlug (1 μl/ml, BD Biosciences). After incubation, cells were surface stained with directly conjugated anti-CD4 mAb and anti-CD62L mAb for 30 min on ice, washed, incubated in Cytofix/Cytoperm solution (BD Biosciences) for 20 min on ice, washed, and stained for IL-2, IFN-γ, or CD40L with directly conjugated Abs on ice for 30 min.

RNA isolation and amplification

WT and CD27−/− OT-II transgenic mice were immunized with OVA plus CT. At the indicated time points, cells from DLN and spleen were sorted

FIGURE 2. CD27 on CD4+ T cells promotes the primary CD8+ T cell response. WT mice of the CD45.1 allotype received WT or CD27−/− OT-II T cells of the CD45.2 allotype or no adoptive transfer, as indicated. At day 2 after transfer, mice were challenged with OVA protein. At the indicated days after immunization, the recipients’ CD8+ T cell responses specific for OVA257–264 peptide in the context of H-2Kb in DLN, spleen, and lung were followed by flow cytometric analysis with MHC tetramers and anti-CD8 mAb. These are the same mice as were analyzed for the CD4+ T cell response in Fig. 1A and statistics are the same as indicated for that figure.

Intracellular staining

To determine IL-2, IFN-γ, and CD40L production by CD4+ T cells directly ex vivo, WT and CD27−/− OT-II transgenic mice were immunized intranasally with OVA protein plus CT. At indicated time points after immunization, single-cell suspensions from spleen were prepared and stimulated with the murine MHC class II I-Aβ binding peptide OVA323–339 (sequence: ISQAVHAAHAEINEAGA, 100 μg/ml) for 5 h at 37°C and 5% CO2 in the presence of recombinant human IL-2 (40 U/ml, Chiron) and GolgiPlug (1 μl/ml, BD Biosciences). After incubation, cells were surface stained with directly conjugated anti-CD4 mAb and anti-CD62L mAb for 30 min on ice, washed, incubated in Cytofix/Cytoperm solution (BD Biosciences) for 20 min on ice, washed, and stained for IL-2, IFN-γ, or CD40L with directly conjugated Abs on ice for 30 min.

RNA isolation and amplification

WT and CD27−/− OT-II transgenic mice were immunized with OVA plus CT. At the indicated time points, cells from DLN and spleen were sorted
Two-tailed Student’s t test indicated significant differences between recipients that received WT or CD27−/− OT-II cells for p = 0.05 (*) and p = 0.01 (**). Data are representative of two experiments.

Results

Contribution of CD27 on CD4+ T cells to primary CD4+ and CD8+ T cell responses

To study the effect of CD27 on the CD8+ helper function of CD4+ T cells, we have used a model of intranasal delivery of OVA protein. This permitted detection of OVA-specific CD4+ and CD8+ T cells and use of OVA-specific OT-II TCR transgenic CD4+ T cells (30). To define how CD27 on Ag-specific CD4+ T cells affected the Ag-specific CD8+ T cell response, we traced the endogenous OVA-specific CD8+ T cell response in mice that had received either WT or CD27−/− OT-II CD4+ T cells. After intranasal delivery of OVA protein, adoptively transferred OT-II CD4+ T cells of the CD27−/− genotype accumulated to a significantly lesser extent than did WT cells in lymphoid organs as well as in the lung, which is the effector site in this model (Fig. 1A). Labeling with CFSE showed that CD27 did not significantly affect cell cycle entry or activity of CD4+ T cells on consecutive days after priming in DLN (Fig. 1B). Most likely, therefore, CD27 supports accumulation of activated Ag-specific CD4+ T cells primarily by promoting their survival, as it does for CD8+ T cells (21). When mice had received adoptive transfer of WT OT-II CD4+ T cells, numbers of endogenous Ag-specific CD8+ T cells in DLN, spleen, and lung at day 8 of the response were higher than in the no transfer situation (Fig. 2). Both WT and CD27−/− OT-II T cells promoted OVA-specific CD8+ T cell accumulation in DLN, but in spleen and lung OVA-specific CD8+ T cell numbers were significantly lower in mice that had received CD27−/− as compared with WT OT-II T cells (Fig. 2). The collective data indicate that CD27 promotes accumulation of Ag-specific CD4+ T cells at priming and tissue sites and suggest that CD27 improves the capacity of CD4+ T cells to support the primary CD8+ T cell response in this protein cross-presentation setting.

CD4+ T cells require CD27 to endow memory CD8+ T cells with the potential for secondary expansion

We next assessed whether CD27 affected the capacity of CD4+ T cells to help the memory CD8+ T cell response in the same...
protein immunization system. To exclude CD27 on CD8+ T cells as a variable, we used CD27−/− mice as recipients for WT or CD27−/− OT-II CD4+ T cells. In this system, endogenous CD4+ and CD8+ T cells lack CD27, and presence or absence of CD27 on adoptively transferred, OVA-specific CD4+ T cells is the only variable. Six weeks after primary immunization with OVA protein, mice were rechallenged with OVA protein and the Ag-specific CD8+ T cell response was analyzed. In mice that had received WT OT-II CD4+ T cells, the memory response of CD27−/− CD8+ T cells was significantly higher in both lung and spleen than in mice that had received CD27+/− CD4+ OT-II T cells (Fig. 3A). In lung, the phenotype was particularly evident, since a large proportion of the CD8+ (effector) T cells at this site stained with H-2Kb/OVA257–264 tetramers. In spleen and DLN, only a small proportion of total CD8+ T cells stained with H-2Kb/OVA257–264 tetramers (Fig. 3A). For spleen, this low frequency of tetramer-stained cells translated to absolute numbers comparable to those in lung. The contribution of DLN to the memory CD8+ T cell response, however, was very small, and no significant difference was detected between WT or CD27-deficient CD4+ T cell help. From these data we conclude that CD27 promotes the capacity of OT-II CD4+ T cells to support accumulation of Ag-specific CD8+ T cells in the memory response.

Steady-state levels of OVA-specific CD27−/− CD8+ T cells at 6 wk after primary challenge were low, but in lung they were significantly higher than in naive mice, indicating the presence of memory (Fig. 3B). We have previously shown that CD27 on CD8+ T cells promotes memory CD8+ T cell formation after influenza virus infection (24). Therefore, the low numbers of memory cells in CD27−/− mice may be in part explained by CD27 deficiency of CD8+ T cells. In this experiment, we sought to compare memory CD8+ T cell formation in CD27−/− mice that received CD27-proficient or -deficient CD4+ T cell help. No significant differences were detected.

Help by CD4+ T cells for the memory CD8+ T cell response is reportedly delivered during the primary response (10). Therefore, we addressed specifically whether CD27 on CD4+ T cells exerted its helper effect before secondary challenge. CD27−/− mice were supplemented with WT or CD27−/− OT-II T cells and immunized with OVA protein. Six weeks later, mice were rechallenged by intranasal application of the MHC class I-restricted immunodominant peptide OVA257–264 to selectively recall OVA-specific CD8+ T cells and not CD4+ T cells. As in the case of rechallenge with OVA protein, accumulation of OVA-specific CD8+ T cells in the lung was significantly improved by delivery of CD27-proficient CD4+ T cell help during the primary response. In spleen, no significant differences were detected at the two time points measured. The absolute contribution of DLN to the memory CD8+ T cells.
cell response was again very small, but in this setting of rechallenge with OVA peptide a significant difference was detected between WT and CD27-deficient CD4+ T cell help (Fig. 4). We conclude that CD27 promotes the capacity of CD4+ T cells to instill memory function into CD8+ T cells during the primary response. This CD27-mediated CD4+ T cell help does not detectably increase formation of CD8+ memory T cells, but it improves their capacity to expand and accumulate at the tissue site after secondary stimulation.

**CD27 costimulation provides CD4+ T cells with CD8+ Th quality**

Given that CD27 promoted the accumulation of Ag-specific CD4+ T cells, its effect on CD4+ T cell help might be explained by a contribution to CD4+ Th cell quantity rather than to quality. To examine whether this was the case, we corrected for the deficiency in CD27−/− CD4+ effector T cell accumulation by adoptively transferring higher numbers of CD27+/− OT-II T cells than WT OT-II T cells in recipient mice. For the primary response, WT recipients were used to recapitulate the experiment depicted in Figs. 1 and 2. After primary immunization with OVA, representation of CD27−/− OT-II CD4+ T cells in blood and spleen was lower than that of WT OT-II CD4+ T cells when equal numbers (0.5 × 10^6) had been transferred, but it was corrected when 4 times as many (2.0 × 10^6) CD27+/− OT-II T cells had been transferred (Fig. 5A). However, in contrast to WT OT-II T cells, CD27−/− OT-II T cells could not support accumulation of endogenous OVA-specific CD8+ T cells in the primary response, independent of their number (Fig. 5A). For the memory response, CD27−/− recipients were used to recapitulate the experiment depicted in Fig. 4. After adoptive transfer of the indicated cell populations, mice were immunized with OVA protein. Sampling of the blood indicated that at day 5, the frequency of CD27−/− OT-II T cells was higher than that of WT OT-II T cells when 2.0 × 10^6 cells had been transferred (Fig. 5B). Memory CD8+ T cells were selectively recalled 6 wk after priming by challenge with OVA257–264 peptide. Responses were read out at day 7 in the spleen. A significant helper effect on secondary expansion was observed for WT OT-II T cells, but not for either low or high numbers of CD27−/− OT-II T cells (Fig. 5B). The helper effect of WT T cells did not concern acquisition of effector function, since the percentage of granzyme B-phenotype was the same in all adoptive transfer situations for both the primary response (data not shown) and the secondary response (Fig. 5C). The experimental setup guarantees that help for secondary expansion of memory CD8+ T cells was delivered during the primary response. We conclude, therefore, that CD27 has a qualitative effect on CD4+ T cells that allows them to increase effector CD8+ T cell numbers in the primary response and to endow CD8+ T cells during priming with the capacity to mount an optimal secondary response.

**CD27 induces a Th1-type gene expression profile in CD4+ T cells**

To monitor the impact of CD27 on CD4+ effector T cell quality in an unbiased manner, we compared genome-wide mRNA expression profiles of WT and CD27−/− OT-II CD4+ T cells. Mice were immunized with OVA and 3 or 4 days later cells were isolated from DLN and spleen. They were sorted for a CD69+ CD62Llow/− CD4+ phenotype to enrich for recently activated effector-type CD4+ T cells (Fig. 6A). Microarray revealed CD27 as the most strongly and consistently differentially expressed gene between WT and CD27−/− CD4+ T cells, confirming the validity of the approach (Fig. 6A). A hierarchy of differentially expressed genes was established, based on the frequency in which differential ex-
pression was observed at the two time points in the two tissues analyzed, as well as on the cumulative measure of differential expression. In this way, we identified 20 gene products that were enriched in activated WT cells, indicating that they are up-regulated by CD27. Importantly, six of these were also identified as differentially expressed genes, while the relevant probes were down-regulated under Th2 differentiation conditions. Moreover, its over-expression was observed at the two time points in the two tissues analyzed (data not shown). IFN-γ production in effector CD4+ T cells was significantly higher in WT than in CD27-/- mice in DLN, spleen, and lung after a brief in vitro restimulation with antigenic peptide. To selectively monitor responder CD4+ T cells, they were gated for a CD62Llow/CD44highCD69low phenotype, but analysis of the total OT-II CD4+ T cell pool gave similar results (data not shown). IFN-γ production in effector CD4+ T cells was detectable from day 4 onward, particularly in spleen and lung (Fig. 7A). On day 6, the frequency of IFN-γ-producing CD4+ effector T cells was significantly higher in WT than in CD27-/- mice in DLN, spleen, and lung.

**Table I. Characteristics of CD27-regulated molecules in CD4+ T cells**

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNFRSF7</td>
<td>TNF receptor superfamily member 7 (CD27)</td>
<td>Receptor for TNFRSF7/CD70, mediates survival of activated T cells</td>
</tr>
<tr>
<td>MSST2</td>
<td>Microsomal glutathione S-transferase 2</td>
<td>Can catalyze production of LTC4 from LTA4 and reduced glutathione, belongs to MAPEG family</td>
</tr>
<tr>
<td>MS4A4B</td>
<td>Membrane-spanning 4 domains, subfamily A, member 4B</td>
<td>Th1-specific protein, enhances IL-2 and IFN-γ production in activated T cells</td>
</tr>
<tr>
<td>IFIT1</td>
<td>IFN-induced protein with tetraoctapeptide repeats 1</td>
<td>IFN-inducible protein, up-regulated in patients with systemic lupus erythematosus or myelodysplasias</td>
</tr>
<tr>
<td>RSAD2</td>
<td>Radical S-adenosyl methionine domain-containing 2</td>
<td>IFN-inducible antiviral protein directly induced by human CMV</td>
</tr>
<tr>
<td>MPO</td>
<td>Myeloperoxidase</td>
<td>Present in polymorphonuclear leukocytes, catalyzes the production of hypohalous acids</td>
</tr>
<tr>
<td>sim. to PYKIN</td>
<td>Similar to pyrin and HIN domain family, member 1 isoform</td>
<td>Ly6 family member</td>
</tr>
<tr>
<td>LTY6C</td>
<td>Lymphocyte Ag 6 complex, locus C</td>
<td>Reversible hydration of CO2</td>
</tr>
<tr>
<td>CAR2</td>
<td>Carbonic anhydrase 2</td>
<td>Close relative of MS4A4B</td>
</tr>
<tr>
<td>MS4A4C</td>
<td>Membrane-spanning 4 domains, subfamily A, member 4C</td>
<td>Unknown</td>
</tr>
<tr>
<td>sim. to IF203</td>
<td>Similar to IFN-activatable protein 203</td>
<td>Unknown</td>
</tr>
<tr>
<td>OAS1</td>
<td>2′-5′-oligoadenylate synthetase 1</td>
<td>Mediates resistance to virus infection</td>
</tr>
<tr>
<td>LY6F</td>
<td>Lymphocyte Ag 6 complex, locus F</td>
<td>Ly6 family member</td>
</tr>
<tr>
<td>ISG20</td>
<td>IFN-stimulated gene 20-kDa protein</td>
<td>Endonuclease with specificity for single-stranded RNA</td>
</tr>
<tr>
<td>HERC5</td>
<td>Hect domain and RLD 5</td>
<td>Ubiquitin ligase</td>
</tr>
<tr>
<td>RNF43</td>
<td>Ring finger protein 43</td>
<td>Negative coregulator for hlm-homodomain transcription factors</td>
</tr>
<tr>
<td>GBP2</td>
<td>Guanylate nucleotide-binding protein 2</td>
<td>Binds GTP, GDP, and GMP</td>
</tr>
<tr>
<td>BAZ2A</td>
<td>Bromodomain adjacent to zinc finger domain, 2A</td>
<td>May play a role in regulating transcription, may regulate chromatin structure of the tRNA locus</td>
</tr>
<tr>
<td>TRIM30</td>
<td>Tripartite motif protein 30</td>
<td>Tripart-acting factor that regulates expression of IL-2R σ-chain</td>
</tr>
<tr>
<td>IFN inducible protein 27</td>
<td>ifn-α-inducible protein 27</td>
<td>Unknown</td>
</tr>
<tr>
<td>CD74</td>
<td>IFN-α-inducible protein 27</td>
<td>Binds to MHC class II αβ heterodimers and transports them to lysosomal compartments</td>
</tr>
<tr>
<td>LAG3</td>
<td>Lymphocyte activation gene 3</td>
<td>Involved in lymphocyte activation (membrane protein)</td>
</tr>
<tr>
<td>PRMT1</td>
<td>Protein arginine N-methyltransferase 1</td>
<td>Methylates HNRNPA1, SUPT5H, and histones</td>
</tr>
<tr>
<td>PGIPEN</td>
<td>RNA-binding protein FUS (Pigpen protein)</td>
<td>Binds both ssDNA and dsDNA, possible role in genomic integrity</td>
</tr>
<tr>
<td>sim. to N. S. E. BP</td>
<td>Similar to nuclease sensitive element-binding protein 1</td>
<td>Unknown</td>
</tr>
<tr>
<td>PLXND1</td>
<td>Plexin D1</td>
<td>Putative receptor involved in the development of neural and epithelial tissues</td>
</tr>
</tbody>
</table>

* Nomenclature and description of these molecules is according to the Swiss-Prot database; information on function is modified from the same source in combination with literature data.

The impact of CD27 on expression of IFN-γ and IL-2 in effector CD4+ T cells was next studied at the protein level. OT-II transgenic mice were challenged with OVA protein, and cytokine production was determined by intracellular staining of CD4+ T cells taken from DLN, spleen, and lung after a brief in vitro restimulation with antigenic peptide. To selectively monitor responder CD4+ T cells, they were gated for a CD62Llow/CD69low phenotype, but analysis of the total OT-II CD4+ T cell pool gave similar results (data not shown). IFN-γ production in effector CD4+ T cells was detectable from day 4 onward, particularly in spleen and lung (Fig. 7A). On day 6, the frequency of IFN-γ-producing CD4+ effector T cells was significantly higher in WT than in CD27-/- mice in DLN, spleen, and lung.
IL-2 production was detectable in effector CD4+ T cells in spleen and lung. At day 6, the frequency of IL-2-producing CD4+ effector T cells in the lung was significantly higher in WT than in CD27−/− mice (Fig. 7B). In an independent experiment, we also found significantly higher IL-2 production by WT CD4+ effector T cells in lung at day 8 (data not shown). Throughout this time course of the primary response, the absolute number of CD4+ effector T cells did not differ between WT and CD27−/− mice (data not shown), confirming that CD27 had a qualitative impact on effector function. At day 6 after secondary challenge, both IFN-γ and IL-2 production by CD27−/− CD4+ T cells was significantly impaired as well (Fig. 7C). In this model of antigenic challenge, CD4+ T cells did not detectably produce IL-4 or IL-10 (data not shown). These data are in agreement with the concept that CD27 instructs Th1-type effector function in CD4+ T cells. Failure to detect IL-2 and IFN-γ as differentially expressed genes in the microarray may be due to the time points chosen for the analysis.

FIGURE 7. CD27 induces expression of IFN-γ and IL-2 in CD4+ T cells. A and B, WT and CD27−/− OT-II transgenic mice were immunized with OVA protein. At the indicated days after primary challenge, cells from spleen, DLN, and lung were stimulated in vitro with OVA323–339 peptide or mock peptide as control and subsequently analyzed for IFN-γ (A) or IL-2 (B) expression in CD62Llow/− effector CD4+ T cells by intracellular staining. Representative FACS plots are from lung samples taken at day 6. Mean fluorescence intensities (MFI) for the relevant molecules are indicated on x- and y-axes. C, Mice were immunized with OVA protein and rechallenged with OVA 6 wk later. At day 6 after the boost, IL-2 and IFN-γ production by CD4+ T cells was detected by intracellular staining. Data points in line and bar diagrams represent mean values + SEM (n = 3–4). Data are representative of multiple kinetics analyses. Two-tailed Student’s t test indicated significant differences between WT and CD27−/− mice for p ≤ 0.05 (*) and p ≤ 0.01 (**).
FIGURE 8. WT or CD27−/− OT-II T cells were adoptively transferred into CD27−/− recipients, which were primed with OVA protein and rechallenged after 6 wk with OVA323–337 peptide to selectively recall OVA-specific CD8+ T cells. At day 5 after peptide challenge, DLN, spleen, and lung were harvested from three mice per group and cells were pooled and sorted by flow cytometry for a H-2Kb/OVA323–337 tetramer−/− CD8+ phenotype. The resulting populations of endogenous Ag-specific CD8+ T cells were used for mRNA isolation. A. Microarray results depicted as dot plots. The comparative setting is mRNA from CD8+ T cells that had received WT- vs CD27-deficient CD4+ T cell help. The A value on the x-axis is a parameter for signal intensity, and the M value on the y-axis gives the ratio of representation in the selected vs the unsampled population. Colors indicate significant differences according to p < 0.00003. The circle indicates the signal for MS4A4B. B. Real-time PCR was performed on the same samples used for microarray analysis, as outlined in the Materials and Methods section, and the relative amount of MS4A4B transcript is expressed as standardized on GAPDH transcript level.

In the same time courses, we examined CD40L expression on CD4+ T cells, since this molecule has been implicated in help for the CD8+ T cell response (13, 17, 19). CD40L was clearly upregulated between day 3 and day 4 after immunization, but no significant differences between WT and CD27−/− OT-II CD4+ T cells were observed (results not shown).

Impact of CD27-proficient CD4+ T cell help on CD8+ memory T cell quality

The next question was how CD27-proficient T cell help affected memory CD8+ T cell quality in terms of gene expression. We found no difference in granzyme B production by recalled memory CD8+ T cells that had received CD27-proficient or -deficient help (Fig. 6C). To approach the same question in an unbiased manner, we performed genome-wide mRNA expression profiling. WT or CD27−/− OT-II T cells were adoptively transferred into CD27−/− recipients, which were primed with OVA protein and rechallenged after 6 wk with OVA323–337 peptide to selectively recall OVA-specific CD8+ T cells. At day 5 after peptide challenge, DLN, spleen, and lung were harvested from three mice per group and cells were pooled, stained with H-2Kb/OVA323–337 tetramers and anti-CD8 mAb, and sorted by flow cytometry. The resulting population of endogenous Ag-specific CD8+ T cells was used for gene expression profiling. The experimental setup ensured that differential gene expression was diagnostic for CD27-proficient vs CD27-deficient CD4+ T cell help. The selection as defined in the Materials and Methods section revealed four genes in DLN that contributed to the capacity of CD4+ T cells to deliver CD8+ T cell help.

Discussion

We have investigated how CD27 affects CD4+ T cell function by using a model of intranasal immunization with OVA protein. CD27 is expressed on the great majority of both CD4+ and CD8+ naive and activated T cells in humans and mice and is expected to make a functional contribution to both subsets (2, 8, 36). Studies in CD27−/− and CD70 transgenic mice have already indicated that CD27/CD70 interactions affect accumulation of CD4+ T cell effector cells in lymphoid organs and tissue sites (23, 26). However, CD27/CD70 interactions do not contribute to the B cell response after virus infection or protein immunization (24, 27, 36), indicating that CD27 on CD4+ T cells is not important for B cell help, at least in the mouse. Our present findings demonstrate that CD27 contributes to the capacity of CD4+ T cells to deliver CD8+ T cell help.

It has been established that CD4+ T cells deliver help for the primary CD8+ T cell response via CD40. CD40L on activated CD4+ T cells can interact with CD40 on DC, thus licensing them to promote CD8+ T cell priming (11–13). Taraban et al. showed recently that blocking Ab to CD70 impeded helper-dependent CD8+ T cell priming in response to protein immunization. These authors suggested that this was due to a direct effect on CD8+ T cells (37). However, we demonstrate herein that the contribution of CD27 to the primary CD8+ T cell response in such a cross-presentation setting includes indirect effects via CD4+ T cell help. We show that CD27 has no effect on CD40L expression on CD4+ T
cells, but perhaps its capacity to promote IFN-γ production contributes to CD8+ T cell priming. IFN-γ stimulates IL-12 production by DC, which in turn can increment CD8+ T cell expansion (38). Further studies will have to demonstrate whether CD27 on CD8+ T cells must interact with CD70 on DC to influence CD8+ T cell fate. Use of CD70 knockout mice, which we have recently generated, will facilitate this work. Note that protein immunization does not reflect conditions of pathogen infection, in which inflammatory conditions lead to optimal DC maturation. However, in support of our findings, a recent study using anti-CD70-blocking Ab showed that CD27 signals on CD4+ T cells prevented apoptosis of CD8+ T cells at the effector phase after influenza virus infection (39).

CD27 on CD4+ T cells did not have a significant effect on CD8+ T cell memory formation in our test situation. However, CD27/CD70 interactions are clearly important for this. Formation of memory CD8+ T cells was reduced in CD27−/− mice (24) and in mice treated with blocking CD70 Ab (37). Conversely, CD8+ effector T cell contraction was reduced in CD70 transgenic mice (25). Two patient studies using adoptive T cell transfer indicated that also in the human system, CD27 expression is beneficial for CD8+ memory T cell formation (40, 41). Presumably, survival signals imparted by CD27 on CD8+ T cells during the contraction phase play a key role in memory CD8+ T cell formation. Down-regulated CD27 expression has emerged as a hallmark of memory CD8+ T cells that constitutively display effector function and are found predominantly in nonlymphoid tissue (42, 43). CD27 down-regulation seems to be part of a functional program rather than the result of interaction with CD70 (43). Memory CD4+ T cells at tissue sites also display this phenotype (44). Its functional significance is presently difficult to envision, but it suggests a particular relevance of CD27/CD70 interactions for T cells at the tissue site, in line with data gathered from CD27−/− mice (36).

Even after bacterial or viral infection when priming conditions are inflammatory, CD4+ T cell help appears to be required for adequate memory CD8+ T cell function (10, 18–20). Pertinent studies show that secondary expansion of memory CD8+ T cells is deficient when CD4+ T cell help has been lacking throughout the primary response. These observations have launched the notion that CD4+ T cell help is required to “program” CD8+ T cells for appropriate secondary responsiveness. A recent study indicated that CD27/CD70 interactions during priming play a role in programming memory CD8+ T cells for secondary expansion (39). The work presented herein argues that CD27 signaling into CD4+ T cells contributes to the functional makeup that enables them to contribute to CD8+ T cell memory programming. Our data do not allow for a conclusion as to whether CD27-dependent CD4+ T cell help has a differential impact on central or effector/memory memory cells. We observed consistently that secondary accumulation of CD8+ T cells at the tissue site (lung) was reduced in case of CD27-deficient CD4+ T cell help. Whether this response was predominantly due to recall of effector memory cells, we cannot say. In the experiments using OVA peptide for recall, we noted a significant impact of CD27-dependent CD4+ T cell help in the DLN, which contains primarily central memory cells (42). Such significant impact was not observed when CD4+ T cells were recalled by OVA protein. However, gene expression profiling revealed that certain CD8+ memory cells residing in lung and spleen, as well as in DLN, had received the imprint of CD27-proficient CD4+ T cell help. To determine whether these include central memory cells, it will be important to use other models of antigenic stimulation, in particular those that are inflammatory and generate large numbers of memory cells, which can more easily be phenotypically and functionally dissected.

We have found that CD27 supports accumulation of effector CD4+ T cells without detectably affecting cell cycle entry or activity at the site of priming. These data argue that CD27 promotes survival of primed CD4+ T cells, as it does for CD8+ T cells (23). It has recently been recognized that contrary to dogma, primed T cells do not require IL-2 for clonal expansion. Although in vitro IL-2 is used as the prototypical cytokine that drives division of newly activated T cells, in vivo T cells enter the cell cycle and go through consecutive rounds of division efficiently in the absence of IL-2 (45, 46). At later stages in the primary response, however, autocrine or paracrine IL-2 supports accumulation of effector cells, particularly in nonlymphoid tissues (45, 46). This points to an important role for IL-2 in promoting effector T cell survival. CD27 was found to stimulate IL-2 production (Fig. 7), as well as IL-2 receptor α-chain expression in CD4+ T cells (results not shown), suggesting that this is a mechanism by which it contributes to effector T cell survival. We saw no effect of CD27 on IL-7 receptor α-chain expression on CD4+ T cells (results not shown).

Our data indicate that the impact of CD27 on effector CD4+ T cell quality rather than quantity is important for the capacity to help the CD8+ T cell response. The effects of CD27 on protein and gene expression indicate that CD27 instructs a Th1-type differentiation program into CD4+ T cells. Effects of CD27 on the frequency of IFN-γ-producing effector cells were consistently observed at late time points (days 5 and 6) in the primary response. Consistently, in CD70 transgenic mice, the frequency of CD4+ and CD8+ T cells producing IFN-γ is strongly increased, as is the level of cytokine production on a per-cell basis (26). These findings tie in with recent observations in human and mouse CD4+ T cells. In the mouse, it was found that there exists an IL-12-independent pathway to induce IFN-γ production in CD4+ T cells, which involves CD70 on DEC205+ DC (28). In human CD4+ T cells, deliberate CD27 costimulation by CD70-transfected cells resulted in transcriptional up-regulation of the T-bet transcription factor and sensitized the cells for IL-12-induced Th1 differentiation (47). In line with Th1 programming, we also observed effects of CD27 on the frequency of IL-2-producing effector cells in the primary response, albeit less pronounced than for IFN-γ. In the memory response, the frequency of IFN-γ- and IL-2-producing CD4+ responder T cells was clearly reduced in the absence of CD27 costimulation. It has been demonstrated that IL-2 is important to program memory CD8+ T cells for secondary expansion (48). Therefore, CD27-regulated IL-2 production may well be an aspect of CD4+ T cell help to memory CD8+ T cell response. CD4+ T cells are not the only source of IL-2 that provides help for the primary T cell response according to a recent study (49), but for the memory response this remains to be examined. A role for CD27-regulated IFN-γ production seems less likely, since IFN-γ was recently shown to impair, rather than enhance, secondary responses of CD8+ T cells (50).

Microarray analysis ex vivo gave a new insight into the effects of CD27 on effector CD4+ T cell quality. CD27 up-regulated expression of MS4A4B and its close relative MS4A4C. These molecules are encoded by a gene cluster, which in humans has been linked to allergy (35). They are CD20-like molecules of unknown function that span the membrane four times. MS4A4B was originally cloned as a gene selectively expressed in Th1 and not Th2 mouse T cells (34). Overexpression of MS4A4B in mouse T cells led to increased IL-2 and IFN-γ production (35), so it will be interesting to see whether certain CD27-mediated effects, such as induction of these cytokines, lie downstream of MS4A4B. A large proportion of CD27-regulated genes were designated to be part of
a network downstream of IFN-γ by the Ingenuity Systems program. However, many of them can also be regulated by other stimuli (Ingenuity Systems), and MS4A4B is induced under Th1 conditions in an IFN-γ-independent fashion (34). Moreover, the microarray analysis did not reveal differential expression of IFN-γ itself at the time points tested, even though the oligonucleotide probe was present. Therefore, the dataset may well include genes that are under direct transcriptional control of CD27.

The fact that CD4⁺ T cell help alters the capacity of memory CD8⁺ to undergo secondary expansion indicates a functional program that can be regulated by CD27. Such regulation or help can be accomplished by epigenetic effects that alter gene accessibility to transcriptional activity. Interestingly in this context is the finding that CD4⁺ T cell help contributes to epigenetic remodeling of the IL-2 and IFN-γ locus in CD8⁺ T cells (51). Our microarray analysis revealed MS4A4B as a molecular marker for CD4⁺ T cells that had experienced CD27-proficient, as opposed to CD27-deficient, CD4⁺ T cell help. We have not found evidence for differential expression of TRAIL, which was reported as a hallmark for memory CD8⁺ T cells, in this dataset. Therefore, the dataset may well include genes that are under direct transcriptional control of CD27.

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Disclosures
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