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J Immunol 2005; 174:1357-1364; doi: 10.4049/jimmunol.174.3.1357
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CD70 Signaling Is Critical for CD28-Independent CD8+ T Cell-Mediated Alloimmune Responses In Vivo

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The inability to reproducibly induce robust and durable transplant tolerance using CD28-B7 pathway blockade is in part related to the persistence of alloreactive effector/memory CD8+ T cells that are less dependent on this pathway for their cellular activation. We studied the role of the novel T cell costimulatory pathway, CD27-CD70, in alloimmunity in the presence and absence of CD28-B7 signaling. CD70 blockade prolonged survival of fully mismatched vascularized cardiac allografts in wild-type murine recipients, and in CD28-deficient mice induce long-term survival while significantly preventing the development of chronic allograft vasculopathy. CD70 blockade had little effect on CD4+ T cell function but prevented CD8+ T cell-mediated rejection, inhibited the proliferation and activation of effector CD8+ T cells, and diminished the expansion of effector and memory CD8+ T cells in vivo. Thus, the CD27-CD70 pathway is critical for CD28-independent effector/memory CD8+ alloreactive T cell activation in vivo. These novel findings have important implications for the development of transplantation tolerance-inducing strategies in primates and humans, in which CD8+ T cell depletion is currently mandatory. The Journal of Immunology, 2005, 174: 1357–1364.

The CD28-B7 and CD154-CD40 pathways have been described as the most significant costimulatory pathways for T cell activation, and their blockade has been shown to regulate both auto- and alloimmune responses in experimental models and patients (1–3). However, studies have indicated that such blockade is ineffective in reproducing truly effective tolerance, especially in stringent models such as skin or islet transplantation in rodents, or transplantation of primates (4–10). In addition, graft vasculopathy or chronic rejection cannot be effectively and reproducibly prevented in vascularized organ transplant models using CD28-B7 or CD154-CD40 pathway blockade alone (8, 11–14). These effects may be due to the action of alternative costimulatory pathways, capable of inducing full T cell activation, or cellular mechanisms that are resistant to CD28 or CD154 blockade, such as effector CD8+ T cells, memory T cells, and NK cells (4, 15, 16). Furthermore, these mechanisms may not be mutually exclusive. Recently, the importance of alloreactive CD8+ T cells in preventing tolerance induction has been appreciated (17). As a result, current tolerance-inducing strategies have to specifically target the alloreactive CD8+ T cells, in general by using depleting mAbs, so as to achieve long-term graft survival in stringent models (4, 8, 18–20).

It has become apparent that a number of novel receptors on T cells and their respective ligands on APC can provide efficient costimulatory signals for T cell activation (16, 21–23). These include members of the CD28-B7 superfamily (such as B7RP1-ICOS, PDL1-PD-1) and members of the TNF-TNFR superfamily, such as CD27-CD70, 41BB-41BB, and CD134L-CD134 (16, 18, 22, 24–28). In this study, we examined the interaction between CD27-CD70 and CD28/CTLA4-B7 pathways in the rejection of vascularized cardiac allografts using a blocking Ab that binds CD70 in wild-type (WT) mice and mice lacking CD28 (CD28−/−). Our results clearly demonstrate that the CD27-CD70 costimulatory pathway is critical for CD28-independent effector/memory alloreactive CD8+ T cell activation, and thus represents a new important target in development of strategies for inducing durable transplantation tolerance.

Materials and Methods

Animals

C57BL/6 (H-2b) (B6), B6.C-H2bm12/KbEg (bm12), BALB/c (H-2k), and BALB/c-background CD28−/− mice were purchased from The Jackson Laboratory, and B6 mice homozygous for the mutation that leads to alynphoplasia (aly/aly) were purchased from Clea. B6-background 2C TCR-transgenic (TCR-tg) mice were originally obtained from Dr. D. Loh (Washington University, St. Louis, MO); ABM TCR-tg mice have been

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described previously (29) and were maintained as a breeding colony in our animal facility. Animals were used at 6–14 wk of age. All animals were housed in accordance with institutional and National Institutes of Health guidelines.

**Heterotopic heart transplantation**

Vascularized heart grafts were transplanted using microsurgical techniques essentially as described by Corry et al. (30). Briefly, the harvested donor heart was placed in 4°C saline until transplantation. The recipient mouse was anesthetized by i.p. injection of 4% chloral hydrate. The donor aorta was sutured to the recipient aorta and the donor pulmonary artery to the recipient inferior vena cava end-to-side using 10-0 suture. Graft function was evaluated by daily abdominal palpation. Rejection was defined as complete cessation of cardiac contractility as determined by direct visualization.

**Measurement of alloantibodies**

Naïve B6 splenocytes (1 × 10⁶) were incubated for 30 min at 4°C with 50 μl of serially diluted sera obtained from naïve BALB/c or B6 mice (controls), BALB/c heart recipients 10–14 after transplantation treated with control Ig or anti-CD70 mAb. Cells were washed twice, incubated with 50 μl of FITC-conjugated anti-mouse IgG or anti-mouse IgG2a (both BD Pharmingen) at 4°C for 30 min, and analyzed by flow cytometry using a FACSCalibur (BD Biosciences) and CellQuest software (BD Biosciences). The percentage of donor cells stained at each serum dilution was calculated using the following equation: (x – spontaneous release) × 100 (/total release – spontaneous release).

**Preparation of memory T cells for recall experiments**

An adoptive transfer model in which a small proportion of allospecific CD8⁺ T cells is engrafted into WT recipients. The CD8⁺ T cells have been isolated from naïve C57BL/6 (CD8⁺) cells present in the spleen and lymph nodes of BALB/c mice by magnetic cell separation by depleting MHC class II⁺ cells followed by positive selection of B2⁺ cells. The CD8⁺ T cells (CD8⁺ 2C) cells present in the enriched naïve and memory populations were then quantified and phenotyped, and their evivo T cell activity was measured as previously described (39). Finally, a population of 5–10 × 10⁶ enriched naïve or memory T cells, with the number of CD8⁺ 2C T cells (10 × 10⁶) was adoptively transferred to splenectomized al/o α/β recipients of BALB/c hearts 2 days after transplantation. At the time of rejection or at 200 days after transplantation in mice that did not reject their grafts, mice were...
sacrificed and CD8⁺ 1B2⁺ CD44high T cells present in the blood were quantified by flow cytometry. The number of retrieved 2C T cells was extrapolated according to the following formula: percentage of CD8⁺ 1B2⁺ CD44high cells × number of cells per milliliter of blood × 2 ml (estimated volume of blood per mouse). In each mouse studied, the number of recovered 2C T cells was compared with the number and phenotype of the 2C T cells that were injected 2 days after transplantation.

**Statistics**

Kaplan-Meier survival graphs were constructed, and the log-rank comparisons of the groups were used to calculate p values. Significant differences between experimental groups in ELISPOT assays and in the transgenic models were analyzed using Student’s t test, or one-way ANOVA if more than two groups were present. Differences were considered to be significant at p < 0.05.

**Results**

**CD70 blockade and graft survival in WT recipients**

Vascularized cardiac grafts from C57BL/6 (B6) donors (H-2b) were transplanted into fully allogeneic WT BALB/c (H-2b) recipients. WT recipients acutely rejected their grafts between 7 and 11 days (mean survival time (MST) = 8.9 ± 0.4 days; n = 7), and this was not significantly different when recipients were treated with control Ig (MST = 9 ± 0.4 days; n = 4). However, recipients treated with anti-CD70 mAb (500 μg on day of transplant and 250 μg on days 2, 4, 6 after transplantation) had significant prolongation of graft survival (MST = 20.3 ± 2.3 days; n = 4; p < 0.0021), but ultimately all grafts were rejected (Fig. 1A). In some models, donor-specific transfusion has been reported to synergize with B7 or CD154 blockade in promoting long-term allograft survival and tolerance (40–42). Administration of 5 × 10⁶ spleen cells in combination with anti-CD70 mAb therapy did not further prolong graft survival over that achieved with anti-CD70 mAb alone (MST = 26.3 ± 1.7 days; n = 4; p < 0.0021 compared with untreated controls, NS compared with anti-CD70 mAb alone) (Fig. 1A).

We then evaluated the effect of CD70 blockade on CD4⁺ and CD8⁺ T cell-mediated allograft rejection using the same model. We treated the recipients with a short course of depleting anti-CD4 or anti-CD8 mAb based on a previously established protocol (27, 34). In WT animals, confirming previously published data (34, 43–45), transient depletion of CD8⁺ T cells resulted in modest but significant prolongation of graft survival (MST = 14.3 ± 0.8 days; n = 6; p < 0.0005 compared with untreated controls) (Fig. 1B), whereas transient CD4 depletion resulted in a more pronounced prolongation of graft survival (MST = 46.8 ± 11.3 days; n = 6; p < 0.0005 compared with untreated controls) (C). Administration of anti-CD70 mAb in combination with CD4 depletion induced indefinite graft survival (MST > 100 days; n = 4; p < 0.005 compared with untreated controls) (Fig. 1C). By contrast, administration of anti-CD70 mAb in combination with CD8 depletion did not promote long-term graft survival (graft survival was comparable to the untreated controls and slightly but significantly shorter compared with anti-CD70 mAb therapy alone; MST = 8.0 ± 0.41 days; n = 4; p < 0.01 compared with anti-CD70 mAb treatment alone) (Fig. 1B). These data demonstrate that the CD27-CD70 costimulatory pathway is primarily used by CD8⁺ rather than CD4⁺ T cells to mediate allograft rejection. In addition, our results suggest that the blockade of the CD27-CD70 pathway may slightly accelerate allograft rejection mediated by CD4⁺ T cells, although the biological significance of this acceleration is unclear.

In all cases, the histological pattern of rejection was typical for that seen in murine cardiac allografts, being acute cellular rejection with varying degrees of cellular atrophy. We did not observe vascular rejection.

**CD70 blockade in CD28⁻/⁻ recipients**

To examine the role of the CD27-CD70 pathway in the absence of CD28 costimulation, we studied the effect of CD70 blockade using CD28⁻/⁻ mice as recipients. Our group (34) and others (46) have previously reported prolonged survival of cardiac allografts in CD28⁻/⁻ mice (MST = 22.6 ± 4.0 days; n = 5; p < 0.001 compared with untreated WT recipients) (Fig. 2A). However, all grafts are eventually rejected, a process that is thought to be mediated predominantly by CD8⁺ T cells (34) and NK cells (47).
CD27-CD70 COSTIMULATION IN ALLOIMMUNITY

We assessed using an ELISPOT assay the frequency of alloreactive Th1/T cytotoxic cell 1 (Tc1) (IFN-γ-producing) and Th2/Tc2 (IL-4-, IL-5-producing) specific alloresponses of whole splenocytes in animals treated with or without anti-CD70 mAb. In WT animals, there was no significant difference between untreated and anti-CD70-treated animals (data not shown). However, in untreated CD28−/− recipients, 14 days posttransplantation, there was a frequency of 662.5 ± 179.5 IFN-γ-producing allospecific cells, 119.5 ± 51 IL-4-producing cells, and 16.5 ± 7.5 IL-5-producing cells per million splenocytes. Following treatment with anti-CD70 mAb, there was a significant reduction (54%) in IFN-γ frequencies (304.25 ± 38.25; p = 0.0286 vs untreated recipients) and an increase in both IL-4 (139 ± 14.2) and IL-5 frequencies (47 ± 4.1 cells/million splenocytes, p = 0.0286 vs untreated mice, of 17 and 184% increases, respectively, Fig. 3). Interestingly, the reduction in IFN-γ frequencies was maintained in long-term surviving (>100 days) CD28−/− recipients (43 ± 4 cells/million splenocytes; p = 0.0286 compared with control and day 14 anti-CD70-treated recipients; Fig. 3A).

Measurement of serum alloantibody

Because CD27-CD70 signaling has been implicated in B cell responses, clonal expansion, and T-B cell collaboration (49, 50), we quantified the alloantibody production in untreated transplant recipients and those receiving anti-CD70 mAb, as described (27). Briefly, donor splenocytes were incubated with sera from the transplanted or naive (control) mice and subsequently with isotype-specific fluorochrome-conjugated secondary Abs. The percentages of allogeneic cells with positive binding above that found using naive sera were calculated. In both WT and CD28−/− recipients, there was a significant reduction in the IgG2a alloantibody production following treatment with anti-CD70 mAb compared with untreated recipients.

Administration of anti-CD70 mAb induced indefinite allograft survival in all CD28−/− recipients (MST > 100 days; n = 6; p < 0.001 compared with untreated CD28−/− recipients), and this effect was completely abrogated by combination with a blocking anti-CTLA4 mAb (MST = 24.8 ± 4.2 days; n = 4; p < 0.002 compared with CD28−/− recipients treated with anti-CD70 mAb alone). (Fig. 2A). By contrast, administration of anti-CTLA4 mAb alone only showed a trend to accelerated allograft rejection in CD28−/− recipients, but this was not statistically significant (MST = 16.2 ± 3.3 days; n = 6; NS compared with untreated CD28−/− recipients) (Fig. 2A). Most importantly, blinded morphological evaluation showed that in two-thirds of recipients with long-term allograft function (n = 6), there was no evidence of chronic allograft vasculopathy (representative examples are shown in Fig. 2, B and C) comparable to what we have previously reported for isograft controls in the same model (48). These results indicate that the CD27-CD70 pathway plays a critical role in development of acute and chronic allograft rejection in the absence of CD28-B7 costimulation, and that induction of graft acceptance following CD70 blockade requires an intact CTLA4-negative signaling pathway.

FIGURE 2. Cardiac allograft survival following CD70 blockade in combination with blockade of CTLA4-negative signaling in CD28−/− recipients. A. All cardiac allografts were rejected in BALB/c-background CD28−/− recipients after marginal delay (MST = 22.6 ± 4.0 days; n = 5; p < 0.001 compared with untreated WT recipients). Anti-CD70 mAb treatment alone induced indefinite allograft survival (MST > 100 days; n = 6; p < 0.001 compared with untreated CD28−/− recipients), whereas anti-CTLA4 mAb alone resulted in accelerated allograft rejection (MST = 16.2 ± 3.3 days; n = 6; NS compared with untreated CD28−/− recipients). Prolongation of allograft survival with anti-CD70 mAb treatment in CD28−/− recipients was abrogated by combination with anti-CTLA4 mAb treatment (MST = 24.8 ± 4.2 days; n = 4; p < 0.002 compared with CD28−/− recipients treated with anti-CD70 mAb alone). B and C, Histo-pathology from one CD28−/− allograft recipient treated with anti-CD70 mAb and whose graft survived >100 days, demonstrating no evidence of chronic allograft vasculopathy by H&E (B) or elastin (C) stain. A pattern of no vasculopathy (0/3 CAV score) was found in 67% of animals with long-term functioning grafts (all ×200).
the untreated control recipients (Fig. 4A), whereas there was no difference in the low levels of measured IgG1 alloantibodies (data not shown). These data are consistent with the observed shift from a Th1/Tc1 to a Th2/Tc2 cytokine profile from the ELISPOT assays described above.

**CD70 blockade does not affect NK cell function**

NK cells have been reported to play an important role in CD28-independent allograft rejection (47). Because the CD27-CD70 pathway has been implicated in the activation of NK cells in vitro (51), we studied the effect of anti-CD70 mAb on NK cytolytic activity in vivo by a standard chromium release assay using YAC-1 targets. In WT mice, we found that anti-CD70 mAb therapy did not decrease NK cytolytic activity alone or in combination with CTLA4Ig (data not shown). Moreover, this was confirmed in CD28−/− mice, which received anti-CD70 mAb therapy (Fig. 4B); recipients that had the most pronounced prolongation of graft survival as indicated above. These data suggest that anti-CD70 mAb used at comparable doses to our transplant studies did not significantly alter NK cell-mediated lysis of target cells ex vivo.

**CD27-CD70 pathway is required for the generation of effector CD8+ T cell responses in vivo**

Our transplant survival data with CD4+ T cell depletion (above) suggest that the CD27-CD70 pathway is important in alloreactive CD8+ T cell activation and effector/memory cell generation. Moreover, early in a MLR, there appears to be a greater up-regulation of CD70 on CD8+ than CD4+ T cells (percentage of CD70-expressing cells, 0–0.5% for CD4 cells, 4–7% for CD8). In naive BALB/c spleens, the percentage of CD8+ T cells that expressed an activated effector phenotype (CD44highCD62Llow) was 12.86 ± 2.3. Ten days following transplantation of B6 hearts into WT BALB/c recipients, the percentage of splenic effector CD8+ T cells increased to 21.46 ± 3.8, whereas in animals treated with anti-CD70 mAb, the percentage was 19.9 ± 3.1, a reduction of only 7.3% (p = NS compared with untreated controls). Using CD28−/− BALB/c recipients, there was a significant diminution (of 27%) in splenic effector CD8+ T cell generation following treatment with anti-CD70 mAb bringing the percentage of effector cells back down toward levels found in naïve CD28−/− mice (naïve CD28−/− animals, 9.6 ± 0.45; Ig-treated controls, 14.9 ± 0.7; vs anti-CD70-treated, 11.4 ± 0.64; p = 0.0095; Fig. 5). These data suggest that CD70 blockade can inhibit the activation of alloreactive CD8+ T cells and, in conjunction with the allograft survival and ELISPOT data, demonstrate that it is most effective in the absence of CD28 signaling.

**CD70 blockade does not diminish alloreactive CD4+ but rather CD8+ T cell responses in vivo**

We have recently developed a new adoptive transfer model system to track the behavior of alloreactive CD4+ T cells, and described the effects of B7 blockade by CTLA4Ig (36) and ICOS-B7h blockade (27) on inhibiting expansion and activation of these cells in vivo. We used the same model to study the effect of CD27-CD70 blockade on alloreactive CD4+ T cells. Following adoptive transfer of TCR-tg CD4+ T cells from anti-bm12 mice, reactive to a mutant class II MHC, I-A<sup>bm12</sup>, expressed in bm12 mice (29, 36, 37). WT B6 recipients were transplanted with bm12 skin grafts, as previously described (36, 37). We tracked the alloreactive CD4+ T cells using specific mAbs to the transgenic TCR V<sub>α</sub>2.1 and V<sub>β</sub>8.1 chains (36). Following CD70 blockade, alloreactive CD4+ T cell expansion was not significantly reduced compared with the control Ig-treated group (Fig. 6A). This is in contradiction to our previously reported results with CTLA4Ig and anti-ICOS mAb therapy, both showing significant decrease in expansion of alloreactive

![FIGURE 4](http://www.jimmunol.org/) **FIGURE 4.** Effect of CD70 blockade on B cell and NK cell effector function. A. Inhibition of IgG2a alloantibody formation in transplanted WT and CD28−/− recipients treated with or without anti-CD70 mAb, demonstrating a significant reduction in both WT and CD28−/− recipients. Donor splenocytes were incubated with sera from the transplanted mice (or naive controls) and subsequently with isotype-specific fluorochrome-conjugated secondaries. Percentages of allogeneic cells with positive binding were calculated. B. Killing of YAC-1 targets by NK cells isolated from CD28−/− mice 2 days after treatment with anti-CD70 mAb or control Ig, demonstrating no effect of CD70 blockade on NK cell cytotoxicity.

![FIGURE 5](http://www.jimmunol.org/) **FIGURE 5.** Effect of CD70 blockade on CD8+ T cell effector function. Flow cytometric dot plots showing the expression of CD44 and CD62L on CD8+ splenocytes. Shown is a representative plot of the percentage of CD8+ cells expressing an effector T cell phenotype (CD44<sup>high</sup>CD62L<sup>low</sup>) from naive CD28−/− mice (A), CD28−/− recipients transplanted with B6 hearts treated with control Ig (B), and CD28−/− recipients transplanted with B6 hearts treated with anti-CD70 mAb, 10 days following transplantation (C). D. Graph summarizing the changes in percentages of CD8+CD44<sup>high</sup>CD62L<sup>low</sup> cells in CD28−/− mice (n = 3–6 per group) demonstrating a significant decrease in effector cells in transplant recipients following anti-CD70 treatment (p = 0.0095, Mann-Whitney U test).
The above data indicate that the CD27-CD70 pathway plays an important role on recall/memory CD8^+ T cell responses to viral Ags (54), but its effects on alloreactive memory cells are unknown. To study the role of the CD27-CD70 pathway in the recall of allospecific memory T cells, we used a recently published adoptive transfer model in which specific memory responses can be studied independent of the primary response of naive T cells (39). In this model, CD8^+ TCR-tg memory T cells from 2C mice were transferred to mice that lack secondary lymphoid organs (splenectomized aly/aly mice, of the same B6 background). These mice are unable to initiate immune responses to a transplanted organ but reject the allograft once effector or memory T cells are provided by adoptive transfer (39, 55). Splenectomized aly/aly mice that received memory T cells 2 days after transplantation rejected their cardiac allografts promptly, whereas those that received memory T cells and anti-CD70 mAb accepted the allografts indefinitely (>200 days) (Fig. 7A). Furthermore, a 6-fold increase in the number of allospecific (2C) CD8^+ memory T cells was observed in the untreated mice following rejection, but this increase was limited to 2- to 3-fold in anti-CD70-treated mice (Fig. 7B). These data indicate that the CD27-CD70 pathway plays an important role in the memory recall response by inhibiting the function and proliferation of alloreactive CD8^+ memory T cells.

### Discussion

This is the first report establishing the in vivo functions of the novel T cell costimulatory pathway CD27-CD70 in alloimmunity.

CD4^+ T cells in this model. These data demonstrate that alloreactive CD4^+ T cell functions are relatively preserved following CD70 blockade in vivo, mirroring the allograft survival results following CD8^+ T cell depletion and anti-CD70 mAb therapy in WT recipients. Furthermore, we confirmed the effect of CD70 blockade in inhibiting alloreactive CD8^+ T cells by adoptive transfer of alloreactive TCR-tg CD8^+ T cells (52, 53). In this model, CD8^+ T cells from 2C TCR-tg B6 mice, specific for H-2L^d, were tracked using the clonotypic 1B2 mAb (38). Adoptively transferred WT B6 recipients were transplanted with BALB/c skin grafts, or left untransplanted as controls. Transplanted mice received control Ig or anti-CD70 mAb according to the above protocol. Ten days following transplantation, there was a significant expansion of the transgene CD8^+ T cells in control Ig-treated mice compared with the adoptively transferred but untransplanted mice, but those receiving anti-CD70 mAb had a significant reduction (of 55%) in the expansion of alloreactive CD8^+ T cells (Fig. 6B).

**CD70 blockade inhibits CD8^+ T cell memory recall**

The above data indicate that the CD27-CD70 pathway plays a key role in CD4^+ T cell-independent activation and proliferation of naive and effector alloreactive CD8^+ T cells in vivo, especially in the absence of CD28 signals. Published data from CD27^−/− mice indicate that the CD27-CD70 pathway plays an important role on recall/memory CD8^+ T cell responses to viral Ags (54), but its effects on alloreactive memory cells are unknown. To study the role of the CD27-CD70 pathway in the recall of allospecific memory T cells, we used a recently published adoptive transfer model in which specific memory responses can be studied independent of the primary response of naive T cells (39). In this model, CD8^+ TCR-tg memory T cells from 2C mice were transferred to mice that lack secondary lymphoid organs (splenectomized aly/aly mice, of the same B6 background). These mice are unable to initiate immune responses to a transplanted organ but reject the allograft once effector or memory T cells are provided by adoptive transfer (39, 55). Splenectomized aly/aly mice that received memory T cells 2 days after transplantation rejected their cardiac allografts promptly, whereas those that received memory T cells and anti-CD70 mAb accepted the allografts indefinitely (>200 days) (Fig. 7A). Furthermore, a 6-fold increase in the number of allospecific (2C) CD8^+ memory T cells was observed in the untreated mice following rejection, but this increase was limited to 2- to 3-fold in anti-CD70-treated mice (Fig. 7B). These data indicate that the CD27-CD70 pathway plays an important role in the memory recall response by inhibiting the function and proliferation of alloreactive CD8^+ memory T cells.
Our data clearly demonstrate that the CD27-CD70 pathway plays a critical role in alloimmune responses independently of the CD28-B7 costimulatory pathway. Blockade of CD70 prolonged cardiac allograft survival significantly in WT recipients, induced indefinite graft survival in all CD28-deficient animals, and abrogated the development of chronic rejection in the majority of these long-term surviving allografts. Indeed, the effects of CD70 blockade in CD28\(^{--}\) recipients mirrors the effects of transient CD8\(^{+}\) T cell depletion in the same model (34), both resulting in indefinite allograft survival. Interestingly, blockade of CTLA-4 signaling was able to override the beneficial effect of CD70 blockade. The interaction between these two pathways requires more detailed investigation. Moreover, in CD28-deficient animals, CD70 blockade diminished the frequencies of IFN-\(\gamma\)-producing Th1 and/or Tc1 alloreactive cells and augmented those of IL-4 and IL-5 (Th2/Tc2) cells, whereas IgG2a alloantibodies were diminished in WT and CD28\(^{--}\) recipients. Taken together, these data demonstrate that the CD27-CD70 pathway is integral to the initiation and perpetuation of Th1/Tc1 alloimmune responses in vivo, especially in the absence of CD28. Moreover, it demonstrates some (possibly indirect) effect on B cells, resulting in diminished alloantibody production. Further work is required to analyze other direct effects on APC function.

The second novel and important conclusion from our data is that the CD27-CD70 pathway is required for CD8\(^{+}\) T cell activation, as judged by the expansion of alloreactive CD8\(^{+}\) T cells expressing an effector phenotype and the persistence of alloreactive CD8\(^{+}\) memory T cells in vivo. These cellular effectors appear to be relatively independent of CD28-B7 costimulation (56), and have proven to be important mechanisms by which allograft rejection is mediated and tolerance prevented (4, 18, 57, 58), necessitating the CD27-CD70 pathway is integral to the initiation and perpetuation of Th1/Tc1 alloimmune responses independently of CD28-B7 costimulation (56), and have proven to be important mechanisms by which allograft rejection is mediated and tolerance prevented (4, 18, 57, 58), necessitating the ligation of CD40 on B cells, by playing a key role in T-dependent B cell responses, and plasma cell differentiation (49).

In addition, two separate groups recently reported that coexpression of CD70 and B7--1 on tumor cells enhances antitumor immune responses (63, 64). Our data clearly demonstrate that CD27-CD70 is also a critical costimulatory pathway for CD8\(^{+}\) T cell-mediated alloimmune responses. Therefore, targeting the CD27-CD70 costimulatory pathway, in combination with other therapies that primarily target CD4\(^{+}\) T cells, may be a highly promising strategy for the induction of durable transplantation tolerance to translate to primates and humans.

Acknowledgments

We thank Karla S. Stenger and Susan P. Shea for their invaluable technical assistance.

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