CD28-Specific Antibody Prevents Graft-Versus-Host Disease in Mice

Xue-Zhong Yu, Sasha J. Bidwell, Paul J. Martin and Claudio Anasetti

J Immunol 2000; 164:4564-4568; doi: 10.4049/jimmunol.164.9.4564
http://www.jimmunol.org/content/164/9/4564
CD28-Specific Antibody Prevents Graft-Versus-Host Disease in Mice

Xue-Zhong Yu,* Sasha J. Bidwell,* Paul J. Martin,*† and Claudio Anasetti2†

The costimulatory molecules B7-1 and B7-2 regulate T cell activation by delivering activation signals through CD28 and inhibitory signals through CTLA4. Graft-vs-host disease (GVHD) is caused by activated donor T cells. Previously, we showed that CD28-deficient donor T cells induced less-severe GVHD than wild-type donor T cells, suggesting that CD28 signals exacerbate GVHD. In this paper we demonstrate that CTLA4 signals attenuate the severity of GVHD. Targeting the CD28 receptor with a specific mAb modulates the receptor in vivo, inhibits donor T cell expansion, and prevents GVHD. CTLA4 signaling was necessary for this effect because treatment with a soluble ligand that blocks binding of B7 to both CD28 and CTLA4 did not prevent GVHD as effectively as anti-CD28 mAb. These results support the current model of T cell costimulation in which CD28 signals amplify GVHD while CTLA4 signals inhibit GVHD, providing evidence that selective targeting of CD28 might be a better therapeutic strategy for inducing immunological tolerance than blocking the ligands for both CD28 and CTLA4. The Journal of Immunology, 2000, 164: 4564–4568.

Materials and Methods

Mice

C57BL/6 (B6), B6.C-H2^bm12^ (bm12), B6.C-H2^bm1 (bm1), (B6 × BALB/c)F1 (C58F1), BALB/c H2-dm2 (dm2), and B6.SJL-Ly5.5^ (PepC^ Pep3^ (B6.Ly5.1) mice were purchased from The Jackson Laboratory (Bar Harbor, ME). (B6 × bm12)F1, (B6 × bm12)F1, and (B6 × dm2)F1 (dm2B6F1) mice were bred at the Fred Hutchinson Cancer Research Center (Seattle, WA). Founders for the 2C transgenic strain were kindly provided by Dr. Dennis Y. Loh (Nippon Roche Research Center, Kamakur-shi, Japan). Homozygous B6 CD28^−⁄− mice were a generous gift of Dr. Craig Thompson (2). 2C CD28^−⁄− mice were generated by intercrossing 2C to CD28^−⁄−. All the mice used in this study were housed in microisolator cages.

The protocol for T cell purification and transplantation has been described in detail (19, 21). CD4^+ and CD8^+ T cells were purified by positive selection using a magnetic cell separation system (Miltenyi Biotech, Auburn, CA). To avoid graft rejection, F1 mice were used as recipients in all experiments. (B6 × bm12)F1 or (B6 × bm12)F1 mice were exposed to 700 cGy from 60Co sources at 20 cGy/min. C58F1 or dm2B6F1 mice were exposed to 750 cGy. Purified CD4^+ or CD8^+ cells from B6 donors were injected via the tail vein into irradiated (B6 × bm12)F1, or (B6 × bm12)F1 recipients, respectively. In some experiments, Ly5.1-congenic recipients were utilized to distinguish donor cells from host cells. Irradiated C58F1 or dm2B6F1 recipients were transplanted with 6–15 × 10^6 purified CD8^+ cells from 2C donors. Within each experiment, all recipients were injected with an identical number of 2C CD8 cells.

Preparation and administration of Abs

Both anti-CD28 (37.51) and anti-CTL4A (9H10) are hamster IgG and were kindly provided by Dr. James Allison (University of California, Berkeley, CA). Murine CTLA4-Ig and control L6-Ig were kindly provided by Dr. Robert Peach (Bristol-Myers Squibb, Princeton, NJ). Control hamster Ig was purchased from IGN Pharmaceuticals (Aurora, OH). All the Abs, unless indicated, were injected i.p. at 100 μg/dose every other day for 14 days starting on the day of the transplant.

Flow cytometry

To detect donor CD4 or CD8 cells, splenocytes were isolated from the recipients and stained with mAbs specific for Ly5.1 (A20-1.7, mouse IgG2a; American Type Culture Collection, Manassas, VA) and CD4 (GK1.5) or CD8 (53-6.7). For detection of 2C donor cells, mAbs specific for CD8 and 2C TCR (1B2) were used. The 1B2 hybridoma was kindly provided by Dr. D. Loh (22), and FcT-conjugated 1B2 was prepared in our laboratory. Other mAbs used in this study included: anti-CD2 (RA3-6B2), anti-CD28 (37.51), anti-CD25 (7D4), anti-CTLA4 (9H10), mouse anti-hamster IgG (192.1), and isotype control Abs. Except where noted, all...
CD4 + B eight doses. Data are shown from one experiment in bm12)F 1 mice were injected with PBS alone as controls without GVHD. Each Ab was injected i.p. at 100 μg/recipient every other day for a total of eight doses. Data are shown from one experiment in A, and two replicate experiments in B.

mAbs used for FACS analysis were obtained from PharMingen (San Diego, CA). To test for CD28 modulation in vivo, freshly isolated splenocytes were incubated with saturating amounts of anti-CD28 mAb or normal hamster IgG for 30 min at 4°C. After washing, the cells were labeled with FITC-conjugated mouse anti-hamster IgG. Intracellular detection of CTLA4 was conducted as previously described (23). Briefly, cells were fixed with 1% paraformaldehyde, permeabilized with 0.3% saponin, and stained with anti-CTLA4 mAb followed by FITC-conjugated mouse anti-hamster IgG. We used a FACScan with CellQuest software (Becton Dickinson, San Jose, CA) for flow cytometric analysis.

Statistical analysis
Continuous distributions were compared by Student’s t tests. Survival distributions were compared by log-rank tests. Two-sided p values <0.05 were considered significant.

Results

CTLA4 signals inhibit GVHD
To determine the effect of CTLA4 signals on the development of GVHD, we first tested whether CTLA4 blockade with a nonstimulatory, bivalent mAb would accelerate GVHD. Sublethally irradiated (700 cGy) (B6 × bm12)F 1 mice were transplanted with purified CD4 + cells from wild-type B6 mice and treated with anti-CTLA4 mAb or hamster IgG at 100 μg/dose every other day for a total of eight doses. Treatment with anti-CTLA4 mAb was shown to accelerate GVHD lethality (p = 0.005) (Fig. 1A).

B7:CTLA4 interaction may inhibit CD28- or TCR-driven intracellular signals. To distinguish between these two possibilities, experiments were performed to test the effect of B7:CTLA4 interaction in development of GVHD induced by CD28-deficient T cells. Sublethally irradiated (B6 × bm12)F 1 mice were transplanted with CD4 + cells from wild-type B6 mice, and the recipients were treated with control Abs or control L6-Ig. CTLA4-Ig treatment significantly accelerated and exacerbated GVHD lethality compared with control treatment (p = 0.00008) (Fig. 1B). These results indicate that the B7:CTLA4 interaction plays a protective role in the development of GVHD independent of CD28, and interference with the B7:CTLA4 interaction enhances GVHD mortality by removing regulatory controls on TCR-driven intracellular responses.

Anti-CD28 mAbs prevent lethal GVHD
Simultaneous blockade of B7 interaction with anti-CD28 and CTLA4 by administration of soluble CTLA4-Ig or B7-specific mAbs can partially inhibit the development of GVHD in mice (9–12). Because CD28 signals enhance GVHD (19, 20), while CTLA4 signals inhibit GVHD (Fig. 1), we reasoned that the severity of GVHD would be decreased by selectively blocking CD28 costimulation while still allowing CTLA4 engagement on donor T cells. We tested the effect of anti-CD28 mAb in preventing GVHD based on the observation that the administration of intact anti-CD28 mAb inhibits T cell expansion in vivo (24–26), even though anti-CD28 mAb amplifies T cell activation in vitro. Sublethally irradiated MHC class II incompatible (B6 × bm12) or MHC class I incompatible (B6 × bm1)F 1 mice were transplanted with B6 CD4 + or CD8 + T cells, respectively. Recipients treated with anti-CD28 mAb, CTLA4-Ig, or hamster IgG plus L6-Ig at 100 μg/dose every other day from day 0 to day 14. Irradiated controls that were not transplanted developed transient pancytopenia, but all recovered and survived longer than 100 days (Fig. 2A and B). Recipients injected with allogenic T cells and treated with control Abs became acutely ill with progressive weight loss, ruffled fur, and kyphosis, and all died at a median of 15 days after transplant. Both CTLA4-Ig and anti-CD28 mAb significantly improved survival as compared with control Abs (p < 0.0001), but anti-CD28 mAb was significantly more effective than CTLA4-Ig (p < 0.01).

Anti-CD28 mAb inhibits donor T cell expansion
To elucidate the mechanisms by which anti-CD28 treatment prevents GVHD, we tested the effects of anti-CD28 mAb on donor T cell activation and expansion. Sublethally irradiated (B6.Ly5.1 × bm12)F 1 mice (five to six mice per group) were transplanted with purified CD4 cells from B6.Ly5.2 donors and treated with anti-CD28 mAb, CTLA4-Ig, or hamster IgG plus L6-Ig. In recipients treated, respectively, with control Abs, CTLA4-Ig, and anti-CD28 mAb, the percentages of CD4 +Ly5.1 + donor T cells in the blood were 18.8 ± 0.3%, 4.4 ± 1.0%, and 1.7 ± 0.4% on day 6, and 52.1 ± 12.2%, 19.1 ± 14.2%, and 7.3 ± 4.2% on day 15. These data suggest that both CTLA4-Ig and anti-CD28 mAb inhibited donor T cell expansion in vivo and that anti-CD28 mAb was significantly more effective than CTLA4-Ig (p < 0.01). In further experiments, we have tested the effect of anti-CD28 mAb on the
expansion of donor T cells in peripheral lymphoid organs. We found that anti-CD28 mAb inhibited donor T cell expansion and was superior to CTLA4-Ig (Fig. 3, upper panels). We also found that anti-CD28 mAb induced CD28 modulation on CD4\(^+\)Ly5.1\(^2\) donor T cells, whereas control Abs and CTLA4-Ig did not have this effect (Fig. 3, lower panels). Inhibitory effects of anti-CD28 mAb on donor T cell expansion and CD28 modulation were also observed in transplantation of B6 CD8 cells into (B6.Ly5.1\(^3\)bm1)F\(_1\) (data not shown).

Treatment with anti-CD28 mAb does not inhibit expression of CD25 and CTLA4

To follow the fate and function of T cells that recognize recipient alloantigen in vivo, we have used a model in which 2C TCR transgenic T cells were transplanted into CB6F1 recipients that express the specific alloantigen L\(_d\). In this model, 2C cells engrafted, expanded, and became effectors leading to extensive destruction of host B cells and double positive thymocytes (21). In additional experiments, we tested the effect of anti-CD28 mAb on activation of 2C cells in CB6F\(_1\) recipients. Sublethally irradiated CB6F\(_1\) mice were transplanted with purified CD8\(^+\) cells from 2C wild-type or 2C CD28\(^-/-\) mice and treated with anti-CD28 mAb or hamster IgG. On day 4, 2C cells in recipient spleen were analyzed for expression CD25 and CTLA4 (Fig. 4). CTLA4 expression was induced in wild-type 2C cells and in CD28\(^-/-\) 2C cells and was not affected by anti-CD28 treatment, indicating that CD28 signals are not needed for activation-dependent expression of CTLA4. Higher level of CD25 expression was induced in wild-type 2C cells than in CD28\(^-/-\) 2C cells, and CD25 expression was not affected by anti-CD28 treatment. These results show that treatment with anti-CD28 mAb did not block early CD28 signaling that is largely required for CD25 expression.

Anti-CD28 mAb selectively inhibits expansion of alloreactive T cells and destruction of host B cells in the recipients

To determine whether anti-CD28 mAb prevents GVHD by depleting CD28\(^+\) T cells in vivo, we transplanted purified CD8\(^+\) 2C T cells into irradiated CB6F\(_1\) recipients. In this experiment, we used L\(_d\) loss mutant dm2B6F\(_1\) recipients as negative controls. Treatment with anti-CD28 mAb had no effect on the number of 2C cells on day 14 in dm2B6F\(_1\) recipients, indicating that this mAb did not deplete resting CD28\(^+\) cells in vivo. Treatment with anti-CD28 mAb decreased the number of 2C cells in CB6F\(_1\) recipients, indicating that anti-CD28 mAb interfered with expansion of donor T cells that recognize recipient alloantigens (Fig. 5A). The number of host B cells was 50-fold higher in CB6F\(_1\) recipients treated with anti-CD28 mAb than in CB6F\(_1\) recipients treated with control Ab, but 0.07-fold lower than in dm2B6F\(_1\) negative controls (Fig. 5B). These results indicate that GVHD was reduced in severity but not completely prevented by treatment with anti-CD28 mAb.

Discussion

In this study, we have investigated the role of CD28 and CTLA4 in the T cell response to alloantigens in vivo by using models of GVHD in sublethally irradiated mice. Under the conditions tested, donor T cells cause damage to the recipient hematopoietic system, resulting in marrow failure. We showed that anti-CD28 mAb is more effective than CTLA4-Ig in the prevention of GVHD, demonstrating that the selective hindrance of CD28 signals is a better approach to achieve transplant tolerance than B7 blockade, as predicted by other investigators (20, 24, 27). We suspect that the
The number of host B cells in CB6F1 recipients treated with anti-CD28 mAb (Fig. 5). dm2B6F1 (Ld-/-) mice were transplanted with 2C CD8 cells and treated with control Ab or with anti-CD28 mAb. On day 14 after the transplant, splenocytes from each recipient were stained for I2B, CD8, and B220 and analyzed by three-color flow cytometry. The results shown are absolute numbers of each population per spleen. The results represent the mean ± SD from two to three mice per group.

The blockade of CTLA4-signals exacerbated GVHD independently of CD28 expression on donor T cells (Fig. 1), and CTLA4 function, would be required for maximum effects.

In summary, our findings provide evidence that selective targeting of CD28 is more immunosuppressive than targeting B7 and CD154, while preserving CTLA4 function, would be an effective strategy to induce transplantation tolerance.

Acknowledgments

We thank Dr. James Allison for providing hybridomas that produce CD28 and CTLA4-specific mAbs, Dr. Robert Peach for providing murine CTLA4-Ig and L6-Ig, and Jennifer Brackensick for her skilful assistance in the preparation of the manuscript.

References


![FIGURE 5. Anti-CD28 mAb selectively inhibits expansion of 2C T cells and destruction of host B cells in CB6F1 recipients. CB6F1, recipients. CB6F1, (Ld-/-) or dm2B6F1, (Ld+) mice were transplanted with 2C CD8+ cells and treated with control Ab or with anti-CD28 mAb. On day 14 after the transplant, splenocytes from each recipient were stained for I2B, CD8, and B220 and analyzed by three-color flow cytometry. The values shown are absolute numbers of each population per spleen. The results represent the mean ± SD from two to three mice per group.](image-url)


