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In Vivo CD86 Blockade Inhibits CD4⁺ T Cell Activation, Whereas CD80 Blockade Potentiates CD8⁺ T Cell Activation and CTL Effector Function

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To address whether a functional dichotomy exists between CD80 and CD86 in naive T cell activation in vivo, we administered anti-CD80 or CD86 blocking mAb alone or in combination to mice with parent-into-F1 graft-vs-host disease (GVHD). In this model, the injection of naive parental T cells into unirradiated F1 mice results in either a Th1 cytokine-driven, cell-mediated immune response (acute GVHD) or a Th2 cytokine-driven, Ab-mediated response (chronic GVHD) in the same F1 recipient. Combined CD80/CD86 blockade beginning at the time of donor cell transfer mimicked previous results seen with CTLA4Ig and completely abrogated either acute or chronic GVHD by preventing the activation and maturation of donor CD4⁺ T cells as measured by a block in acquisition of memory marker phenotype and cytokine production. Similar results were seen with selective CD86 blockade; however, the degree of CD4 inhibition was always less than that seen with combined CD80/CD86 blockade. A more striking effect was seen with selective CD80 blockade in that chronic GVHD was converted to acute GVHD. This effect was associated with the induction of Th1 cytokine production, donor CD8⁺ T cell activation, and development of antihost CTL. The similarity of this effect to that reported for selective CTLA4 blockade suggests that CD80 is a critical ligand for CTLA4 in mediating the down-regulation of Th1 responses and CD8⁺ T cell activation. In contrast, CD86 is critical for the activation of naive CD4⁺ T cells in either a Th1 or a Th2 cytokine-mediated response. The Journal of Immunology, 2002, 168: 3786–3792.

A ctivation of naive T cells requires cognate interaction of the TCR with Ag as well as a second costimulatory signal. The best-characterized costimulatory molecules are CD28 and CTLA4 on the T cell and their ligands CD80 (B7-1) and CD86 (B7-2), expressed primarily on APCs. The importance of this costimulatory pathway in T cell activation is supported by in vitro data demonstrating that combined CD80 and CD86 blockade at the time of TCR engagement blocks T cell production of IL-2, IFN-γ, and IL-4. Similarly, in vivo studies have shown that combined CD80 and CD86 blockade abrogates naive T cell activation and has proven to be beneficial in a variety of animal disease models in which T cell activation is critical, e.g., lupus, diabetes, experimental autoimmune encephalomyelitis, and allograft rejection (5–11). As a result, CD80 and CD86 have emerged as potential therapeutic targets in diseases mediated by T cells.

Although controversial, it has been suggested that CD80 and CD86 provide distinct signals for the differentiation of T cells (Th0) into either a Th1 (IFN-γ-, IL-2-producing) or a Th2 (IL-4-, IL-10-producing) cytokine phenotype (12). Results using selective CD80 or CD86 blockade in vivo have been contradictory. For example, Kuchroo et al. (13), using experimental autoimmune encephalomyelitis as a model of Th1-mediated disease, demonstrated that selective CD80 blockade ameliorated disease by shifting the cytokine phenotype to a Th2 pattern, whereas selective CD86 blockade worsened disease. It was concluded that in vivo Th1 responses require CD80 costimulation, whereas Th2 responses require CD86 costimulation. By contrast, Lenschow et al. (6), using the nonobese diabetic mouse, a model in which a Th1 phenotype predominates, observed that selective CD80 blockade worsened disease, whereas selective CD86 blockade had a beneficial effect, leading to the conclusion that CD86 was a major costimulatory ligand in a Th1 response. Although disease in both models is Th1 driven, the models differ in several important areas, among which are the use of adjuvants and the degree to which disease is mediated by naive and memory T cells. It is becoming increasingly clear that differences exist in the dependence of memory and naive T cells on CD28 costimulation (14), and that recently identified costimulatory receptor-ligand pairs are important in the differentiation of previously activated T cells (15). However, CD80 and CD86 remain the major costimulatory molecules in the induction of a naive T cell response.

To address whether a functional dichotomy exists between CD80 and CD86 in naive T cell activation in vivo, we used the parent-into-F1, (P→F1)³ model of graft-vs-host disease (GVHD). In this model, the injection of homozygous (parental) naive T cells into unirradiated F1 mice results in either a Th1 cytokine-driven, cell-mediated immune response (acute GVHD) or a Th2 cytokine-driven, Ab-mediated response (chronic GVHD). Important features of the model are as follows: 1) the alloantigen-specific donor
T cells that drive disease can be studied separately from nonspecifically activated (host) T cells; 2) either a Th1-mediated or Th2-mediated response can be induced in the same F1 recipient depending on the strain used for donor cells; and 3) in vivo manipulations that alter disease by blocking T cell activation can be readily distinguished from those that induce immune deviation. Our results indicate that CD86 is critical for naive CD4 T cell activation and differentiation into either a Th1 or Th2 phenotype. In contrast, CD80 is important in mediating a down-regulatory effect on CD8 CTL development, perhaps through preferential binding to CTLA4.

Materials and Methods

Mice

Six- to 8-wk-old C57BL/6 (B6), DBA/2 (DBA or D2), and B6D2F1 mice were obtained from The Jackson Laboratory (Bar Harbor, ME).

Induction of GVHD

Single cell suspensions of splenocytes were obtained from either B6 or DBA male mice and resuspended in RPMI 1640 medium without FCS at 10^6 viable cells per milliliter. Unless otherwise noted, acute and chronic GVHD were induced by tail vein injection of either 50 × 10^6 B6 or 80 × 10^6 DBA splenocytes into nonirradiated B6D2F1 mice, as previously described (16). Negative controls consisted of age- and sex-matched un.injected F1 mice.

In vivo reagents and treatment protocol

Anti-CD80 mAb (16-10A1) and anti-CD86 mAb (GL1) were obtained from BD PharMingen (San Diego, CA). The anti-CD80-specific fusion protein Y100F (17) was a gift of R. Peach (Bristol-Myers Squibb, Princeton, NJ). Reagents were used at a dose of 100 μg of anti-CD80 mAb (16-10A1), 200 μg of Y100F, and 100 μg of anti-CD86 (GL1) mAb. Control mice received 100 μg of rat IgG2a κ (isotype control for anti-CD86) and either 100 μg of hamster IgG (control for anti-CD80 mAb) or 200 μg of L6, a mouse-human fusion Ab specific for L6 tumor Ag (control for Y100F). Reagents were administered i.v. on the day of parental cell transfer and on days 3 and 7.

Flow cytometry studies

At the specified time points mice were sacrificed and spleens were harvested. Splenocytes were first incubated with anti-murine FcγR mAb 2.4G2 (18) for 15–20 min, then stained with saturating concentration of FITC-conjugated, PE-conjugated, or biotin-conjugated mAb. Fluorochrome-conjugated anti-CD4, anti-CD8, anti-B220, anti-H-2K^b, anti-H-2K^d, and anti-CD44 were purchased from BD PharMingen. Three-color flow cytometry was performed using a FACSscan flow cytometer (BD Immunocytometry Systems, San Jose, CA). Lymphocytes were gated based on forward and side scatter. Donor CD4^+ and CD8^+ T cells were identified as cells staining positive for the respective T cell marker and negatively for MHC class I of the nondonor parent. Analysis of CD44 brightness was performed on donor-gated CD4^+ and CD8^+ T cells. Anti-CD44 staining gave a clearly distinguishable bimodal pattern, allowing separation of donor T cells into bright (CD44^high) and dull (CD44^low) subpopulations.

Serologic assays

Serum was tested by ELISA for the presence of anti-ssDNA IgG Abs (16). Briefly, microtiter plates were coated with heat-denatured salmon sperm DNA, blocked with 2% BSA-PBS, and then incubated with serial 2-fold dilutions of mouse serum beginning at a dilution of 1/40. Wells were then washed and incubated with anti-mouse IgG conjugated with alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL). OD was determined at 405 nm. MRL/lpr serum was assayed as a standard, and arbitrary units were calculated using a value of 1000 U/ml for pooled MRL/lpr serum.

Detection of CTL activity ex vivo

Effector CTL activity was tested using freshly harvested splenocytes without an in vitro sensitization period in a 4-h ^3^H release assay as described (19). Targets were 32^P-labeled EL-4 (H-2^b) or P815 (H-2^k) cell lines. Using serial dilutions, effectors were tested in triplicate at four E:T ratios beginning at 100:1 (1.5 × 10^6 effectors and 0.015 × 10^6 targets per well). The percentage of lysis was calculated according to the following formula: [(cpm sample – cpm spontaneous)/(cpm maximum – cpm spontaneous)] × 100%. Results are shown as the mean percent of lysis ± SEM at a given E:T ratio for each treatment group.

Cytokine expression by RT-PCR

The coupled RT-PCR was used as previously described (20). Briefly, 1 × 10^5 splenocytes were homogenized in RNA-Stat-60 (Tel-Test, Friendswood, TX). RNA was reverse-transcribed with Moloney murine leukemia virus reverse transcriptase (Life Technologies, Grand Island, NY). IL-4- and IFN-γ-specific primers were used as previously described (20). To ensure that equal amounts of mRNA were amplified, RT-PCR was performed using primers for the housekeeping gene hypoxanthine phosphoribosyl transferase. For each PCR product the optimal number of PCR cycles was determined experimentally. PCR products were separated on agarose gel, transferred to nitrocellulose, and probed with cytokine-specific probes conjugated with HRP (Oligos Etc., Wilsonville, OR). Blots were developed by ECL (Amersham, Little Chalfont, U.K.). Bands were imaged by autoradiography and quantitated by densitometry. Cytokine densitometry results for each sample were normalized to hypoxanthine phosphoribosyl transferase and results for each cytokine were calculated as fold increase over the respective cytokine expression in control F1 mice according to the ratio (normalized experimental group mean:normalized untreated F1 mean).

Statistical analysis

Data were examined for normality and equal variance (Kolmogorov-Smirnov). If satisfactory, groups were compared by a two-tailed Student’s t test; if not, they were compared by the Mann-Whitney rank sum test.

Results

Maximal inhibition of chronic GVHD-associated B cell expansion is achieved with selective CD80 blockade

To determine the respective roles of CD80 and CD86 in the development of an in vivo Th2-driven response (e.g., chronic GVHD), combined or selective CD80 and CD86 blockade was initiated at the time of parental cell transfer, followed by analysis at day 14. As shown in Table I, untreated or control Ig-treated chronic GVHD mice exhibit the expected ≥2-fold increase in host B cell numbers compared with normal F1 mice, similar to previous reports (21). Significant reductions in B cell expansion were seen for all GVHD groups with CD80 and/or CD86 blockade (p < 0.01), with the strongest effect seen with CD80 blockade. Selective CD80 blockade reduced GVHD-associated B cell expansion by 40–60% compared with untreated or control Ig-treated chronic GVHD. Further inhibition was seen with combined CD80/CD86 blockade, nearly normalizing host B cell numbers (p = 0.03, normal F1 vs CD80/86 blockade). The most striking results were seen for selective CD80 blockade, in which B cell numbers were reduced to ~50% below those of normal F1 mice (p < .001), suggesting that CD80 blockade may not just prevent B cell expansion but may actually promote B cell elimination. This observation has been confirmed in three additional independent experiments (B cell numbers range 15–50% below normal F1 controls) and was seen regardless of whether CD80 blockade was achieved using Y100F or anti-CD80 mAb (data not shown). Although the reduction in B cell expansion following combined CD80/CD86 blockade was typically greater than that seen with CD86 blockade alone, B cell numbers were never reduced below those of normal F1 mice as seen with selective CD80 blockade. Normal F1 mice treated with anti-CD80 mAb alone using the same dosing regimen exhibited no reduction of B cells at day 14 compared with untreated F1 mice (data not shown).

Only combined CD80/CD86 blockade completely inhibits autoantibody production

Serum anti-ssDNA levels were used as a marker for the polyclonal B cell hyperactivity characteristically present in chronic GVHD mice and transiently in acute GVHD mice (16). Selective CD80 or
CD86 blockade resulted in a significant, but incomplete, reduction of serum anti-ssDNA titers compared with control Ig-treated mice (p < 0.01) (Table I). Complete normalization of anti-ssDNA levels was only seen with combined CD80/CD86 blockade (p < 0.05, combined CD80/CD86 blockade vs CD86 or CD80 blockade alone). Taken together, our results with combined CD80/CD86 blockade are similar to those reported for CTLA4Ig (22) and indicate that, as shown in Table I, selective CD86 blockade reduced engraftment of donor CD4 T cells by ~40% compared with control Ig-treated chronic GVHD; however, combined CD80/CD86 blockade resulted in a nearly 50% further reduction in donor CD4+ T cell engraftment (p < 0.05, average additional reduction of 50% over four experiments for CD86 vs CD80/86), implying that both CD80 and CD86 contribute to donor T cell engraftment and expansion. In contrast, no reduction of donor CD4+ T cell engraftment was seen following selective CD80 blockade, but rather an increase in engraftment was observed (range of 20–80% increase in CD4+ T cell engraftment with CD80 blockade compared with untreated chronic GVHD; n = 3 experiments). Strikingly, donor CD8+ T cell engraftment was increased 8-fold compared with control Ig-treated or untreated chronic GVHD mice (Table I) and was seen using either anti-CD80 mAb or Y100F in three additional experiments (range, 3- to 10-fold increase; data not shown).

CD86 blockade inhibits but CD80 blockade promotes donor T cell engraftment

As shown in Table I, selective CD86 blockade reduced engraftment of donor CD4+ T cells by ~40% compared with control Ig-treated chronic GVHD; however, combined CD80/CD86 blockade resulted in a nearly 50% further reduction in donor CD4+ T cell engraftment (p < 0.05, average additional reduction of 50% over four experiments for CD86 vs CD80/86), implying that both CD80 and CD86 contribute to donor T cell engraftment and expansion. In contrast, no reduction of donor CD4+ T cell engraftment was seen following selective CD80 blockade, but rather an

CD80 blockade converts chronic GVHD to acute GVHD

The enhanced donor CD8+ T cell engraftment and reduced host B cell numbers following selective CD80 blockade suggest that CD8+ donor antihost CTL are present and that acute GVHD has developed in these mice. Key features that differentiate acute from chronic GVHD are the presence of ex vivo antihost CTL activity and elevated IFN-γ production, both of which are present in acute GVHD and absent in chronic GVHD (21). As shown in Fig. 1, chronic GVHD mice receiving selective CD80 blockade exhibit significant antihost CTL activity ex vivo, which is not observed for either untreated or control mAb-treated chronic GVHD mice (CD80 blockade vs control Ig-treated, p < 0.01; normal F1 vs chronic GVHD or chronic GVHD plus L6, p = NS). Results in two additional experiments yielded a range in the percentage of specific killing of 10–27.4% with CD80 blockade vs 2–9.2% for control mice (n = 15 mice per treatment). Also consistent with acute GVHD, CD80 blockade in chronic GVHD mice resulted in a significant increase (~4-fold) in IFN-γ mRNA compared with all other groups (p < 0.05) (Fig. 2B). By contrast, the increased IL-4 expression typical of chronic GVHD (21) was absent in anti-

![FIGURE 1](http://www.jimmunol.org/Downloadedfrom/75711207)

**FIGURE 1.** Selective CD80 blockade in chronic GVHD mice induces antihost cytolytic activity. Groups consisted of untreated normal F1 or chronic GVHD mice receiving either no mAb, 200 μg of Y100F, or 200 μg of control mAb (L6) on days 0, 3, and 7 after parental cell transfer (n = 4–5 mice per group). Ex vivo antihost CTL activity was determined on day 10. Results are shown as group mean ± SE percentage of killing on H-2b targets at a given E:T ratio; *, p < 0.01.

![FIGURE 2](http://www.jimmunol.org/Downloadedfrom/75711207)

**FIGURE 2.** Selective CD80 blockade promotes IFN-γ mRNA expression in chronic GVHD mice. Semiquantitative RT-PCR was performed on cDNA from splenocytes taken on day 14 from chronic GVHD mice treated as outlined in Table I. Results are shown as average fold increase over un.injected F1 mice for IL-4 (A) and IFN-γ (B) (n = 5 mice per group).
CD80-treated mice (Fig. 2A). CD86 blockade and combined CD80/CD86 blockade were equally effective in blocking IL-4 production in chronic GVHD mice and did not result in the induction of IFN-γ.

Donor T cell activation in chronic GVHD is inhibited by CD86 blockade but enhanced by CD80 blockade

The foregoing data strongly suggest that CD86 blockade either alone or combined with CD80 blockade inhibits donor T cell activation, whereas selective CD80 blockade does not inhibit T cell activation but rather induces immune deviation. To address the activation status of donor T cells following costimulatory blockade, donor T cell expression of an activation marker, CD44, was assessed on day 14 after cell transfer, and that complete costimulatory blockade with CTLA4Ig treatment precluded donor T cell activation, expansion, and acquisition of memory phenotype (22). As shown in Fig. 3, combined CD80/CD86 blockade in chronic GVHD mice acts similarly to published results with CTLA4Ig in that it completely inhibits the increase in both the percentage and the number of CD44<sup>high</sup> donor CD<sup>+</sup> T cells (p < 0.01). Smaller but statistically significant reductions in the percentage and number of donor CD44<sup>high</sup>CD8<sup>+</sup> T cells were also observed with selective CD86 blockade (p < 0.01, CD86 blockade vs control Ig). In contrast, selective CD80 blockade significantly increased not only the number of CD44<sup>high</sup> donor CD<sup>+</sup> T cells (p < 0.01) but also the number of CD44<sup>high</sup> donor CD<sup>8</sup> T cells compared with untreated chronic GVHD mice, consistent with enhanced donor CD4<sup>+</sup>CD8<sup>+</sup> T cell expansion and CTL maturation induced by selective CD80 blockade. The few CD<sup>8</sup><sup>+</sup> T cells that engraft in untreated chronic GVHD display an activated phenotype that is blocked by CD86 blockade and combined CD80/CD86 blockade.

CD80 and/or CD86 blockade in acute GVHD mimics the effects seen in chronic GVHD

To determine whether the strikingly different effects of selective CD80 and CD86 blockade seen in a Th2/Ab-mediated response (chronic GVHD) are also seen in a Th1/cell-mediated response, selective costimulatory blockade was produced in acute GVHD mice. As shown in Table II, using 50 × 10<sup>6</sup> B6 donor splenocytes, combined CD80/CD86 blockade markedly impaired donor CD<sup>+</sup> and CD8<sup>+</sup> T cell engraftment and completely blocked the elimination of host B cells, characteristic of acute GVHD. These results are similar to those reported for CTLA4Ig treatment in acute GVHD (22, 25) and indicate that, taken together with the above results, combined CD80/CD86 administration is capable of complete costimulatory blockade of donor T cells in either a Th1- or Th2-driven response. Selective CD86 blockade was only marginally effective in blocking acute GVHD, as shown by a small but statistically significant improvement in host B cell survival compared with control Ig-treated acute GVHD mice (8.6 × 10<sup>6</sup> vs 4.5 × 10<sup>6</sup>; p < 0.05).

No inhibition of acute GVHD was seen with selective CD80 blockade, and instead low-level potentiation of disease was observed as measured by small but statistically significant further reduction in host B cells (compared with control Ig-treated or untreated acute GVHD (p < 0.05)). Because acute GVHD induced with 50 × 10<sup>6</sup> donor cells results in near maximal elimination of host B cells, potentiation is difficult to assess. A second experiment was then performed using fewer donor cells (40 × 10<sup>6</sup>) to determine whether a modulatory effect of selective CD80 or CD86 blockade could be detected. As shown in Table II, despite the

<table>
<thead>
<tr>
<th>Group</th>
<th>CD4&lt;sup&gt;+&lt;/sup&gt;</th>
<th>CD8&lt;sup&gt;+&lt;/sup&gt;</th>
<th>B cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal F&lt;sub&gt;1&lt;/sub&gt;</td>
<td>ND&lt;sup&gt;x&lt;/sup&gt;</td>
<td>ND</td>
<td>41.8 ± 2.6</td>
</tr>
<tr>
<td>aGVHD</td>
<td>1.4 ± 0.2</td>
<td>2.3 ± 0.3</td>
<td>2.9 ± 0.3</td>
</tr>
<tr>
<td>aGVHD + Y100F</td>
<td>0.9 ± 0.1</td>
<td>1.5 ± 0.1&lt;sup&gt;y&lt;/sup&gt;</td>
<td>2.4 ± 0.4</td>
</tr>
<tr>
<td>aGVHD + anti-CD86</td>
<td>0.6 ± 0.2</td>
<td>1.4 ± 0.4&lt;sup&gt;y&lt;/sup&gt;</td>
<td>8.6 ± 1.3&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>aGVHD + Y100F/anti-CD86</td>
<td>0.2 ± 0.03&lt;sup&gt;x&lt;/sup&gt;</td>
<td>0.2 ± 0.03&lt;sup&gt;x&lt;/sup&gt;</td>
<td>45.3 ± 4.1&lt;sup&gt;y&lt;/sup&gt;</td>
</tr>
<tr>
<td>aGVHD + control Ig</td>
<td>0.9 ± 0.1</td>
<td>3.1 ± 0.4</td>
<td>4.5 ± 0.4</td>
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</table>

<sup>x</sup> Acute GVHD (aGVHD) was induced by injecting either 50 × 10<sup>6</sup> (Expt. 1) or 40 × 10<sup>6</sup> (Expt. 2) B6 splenocytes. Dosing of mAb is as in Table I and Materials and Methods. Mice were sacrificed on day 14 and lymphocyte subsets were analyzed by flow cytometry. Results are expressed as the mean ± SE. n = 5 mice per group.

<sup>y</sup> ND, Not detectable above background.

<sup>z</sup> Values of p < 0.05 vs untreated acute GVHD.
reduced number of donor cells, near total elimination of host B cells was still observed for untreated or control Ig-treated acute GVHD mice. Nevertheless, acute GVHD was blocked not only by combined CD80/CD86 blockade but also by selective CD86 blockade, as evidenced by complete inhibition of host B cell elimination (Table II), inhibition of IFN-γ up-regulation (Fig. 4), and an inhibition of CD44 up-regulation on donor CD4+ and CD8+ T cells as measured by either percentage or absolute number (Fig. 5), although selective CD86 blockade was less effective in blocking CD44 up-regulation than combined CD80/CD86 blockade. A potentiating effect of selective CD80 blockade was again difficult to detect due to the profound elimination of host B cell, even at the reduced donor cell inoculum, and is better seen in the chronic GVHD model. It should be noted that detection of donor T cells by flow cytometry becomes difficult after 2 wk in untreated acute GVHD mice, due to an overall down-modulation of MHC expression (26) and/or acquisition of host MHC by donor cells (27) that parallels disease severity (28). We interpret the reduced numbers of donor CD8+ T cells at 2 wk in anti-CD80 mAb-treated mice (compared with control Ig-treated or untreated acute GVHD) to be a reflection of accelerated GVHD-associated MHC down-regulation rather than impaired engraftment and milder disease. In support of this, other parameters of acute GVHD such as host B cell elimination (Table II), IFN-γ gene expression (Fig. 4), and percentage of donor CD4/CD8high cells are either comparable to untreated acute GVHD or significantly worse (e.g., host B cells, p < 0.05). Importantly, the observation that IFN-γ levels are not reduced in anti-CD80-treated acute GVHD compared with untreated acute GVHD mice supports our interpretation that donor T cells are not, in fact, reduced by anti-CD80 treatment but rather MHC down-regulation is accelerated and, by extension, acute GVHD is accelerated.

Discussion

Recently, new members of the B7 costimulatory family and their receptors have been described in addition to CD80 and CD86 (15, 29, 30). However, our results underscore the critical importance of CD80 and CD86 in the activation of naive CD4+ T cells. In this report, we have used the P→F1 model of GVHD to determine the in vivo role of CD80 and CD86 in the initiation of either a Th1/ cell-mediated response (acute GVHD) or a Th2/Ab-mediated response (chronic GVHD). Advantages of this model are as follows: 1) either form of GVHD can be induced in the same F1 depending on the T cell subsets injected (16, 31, 32); 2) the Ag-specific T cells driving disease can be monitored separately from the remainder of the T cell pool; and 3) in vivo manipulations that alter disease by blocking T cell activation can be readily distinguished from those that cause alterations in the phenotype of the immune response (immune deviation). Using this model, we observed that combined CD80/CD86 blockade completely inhibited donor CD4+ T cell expansion, cytokine production, and acquisition of an activation phenotype in both acute and chronic GVHD. CD28/B7 costimulation has been reported to be critical for naive T cell differentiation for Th2 cells but not necessarily for Th1 cell development (33, 34); however, in the data presented in this work, combined CD80/CD86 blockade completely blocked both Th1-driven acute GVHD and Th2-driven chronic GVHD. Moreover, our results are in agreement with previous studies demonstrating that naive T cell activation can be prevented by blockade of both CD80 and CD86 using either CTLA4Ig, a fusion protein that binds CD80 and CD86 with high affinity (35), or combined anti-CD80 and anti-CD86 mAb. For example, combined anti-CD80/CD86 mAb treatment has been shown to inhibit acute lethal GVHD in an irradiated recipient model following transfer of either purified CD4+ or CD8+ T cells (36) and to block the Ag-specific expansion and activation of adoptively transferred TCR-transgenic CD4+ T cells (37). Similarly, CTLA4Ig was reported to completely inhibit both acute and chronic GVHD in the P→F1 model (22, 25). The mechanism involved appeared to be the induction of anergy rather than clonal deletion, because donor T cells were not deleted following CTLA4Ig treatment; however, they did not produce IL-2, nor did they acquire an activation phenotype (CD44high) (22).

Our studies with selective costimulatory blockade underscore the dichotomous roles of CD28 and CTLA4, as well as their preferential interactions with CD80 or CD86. CD28 is constitutively expressed on T cells and upon engagement with CD80 and CD86 delivers a proliferative signal to Ag-specific T cells, whereas CTLA4 is not expressed on resting T cells but instead is induced upon activation and delivers a down-regulatory signal to proliferating T cells (38, 39). The critical role of CD28 in donor T cell activation has been reported by Yu et al. (40), whose study demonstrated that anti-CD28 mAb treatment prevented acute GVHD in...
an irradiated recipient model. Our results demonstrating that selective CD86 blockade is almost as effective as combined CD80/86 blockade in blocking donor T cell activation in either form of GVHD, whereas selective CD80 blockade has no detectable inhibitory effect, indicate that in the F1→F1 model of chronic GVHD is usually not sufficient to convert chronic GVHD to acute GVHD (53). Thus, selective CD80 blockade probably promotes CD8+ T cell expansion through both direct and indirect effects.

This study supports the primary role of CD86 in the differentiation of both Th1 and Th2 responses and is the first to show that selective CD80 blockade results in de novo expansion and maturation of CD8+ T cells from inactive precursors, thereby converting chronic GVHD (Th2 mediated) to acute GVHD (Th1 mediated).

These results suggest a potential novel therapeutic role for selective CD80 blockade. Currently, CTLA4 block is being evaluated for its therapeutic potential as an antitumor agent (39). A similar approach with selective CD80 blockade may be possible either alone or in combination with CTLA4 blockade.

References


COSTIMULATORY BLOCKADE IN GVHD


