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*J Immunol* 2000; 165:1111-1118; doi: 10.4049/jimmunol.165.2.1111

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CD40-CD40 Ligand-Independent Activation of CD8+ T Cells Can Trigger Allograft Rejection1

Nick D. Jones,2 Andre Van Maurik, Masaki Hara,3 Bernd M. Spriewald, Oliver Witzke, Peter J. Morris, and Kathryn J. Wood

In experimental transplantation, blockade of CD40-CD40 ligand (CD40L) interactions has proved effective at permitting long-term graft survival and has recently been approved for clinical evaluation. We show that CD4+ T cell-mediated rejection is prevented by anti-CD40L mAb therapy but that CD8+ T cells remain fully functional. Furthermore, blocking CD40L interactions has no effect on CD8+ T cell activation, proliferation, differentiation, homing to the target allograft, or cytokine production. We conclude that CD40L is not an important costimulatory molecule for CD8+ T cell activation and that following transplantation donor APC can activate recipient CD8+ T cells directly without first being primed by CD4+ T cells. The Journal of Immunology, 2000, 165: 1111–1118.

CD40 ligand (CD40L)4 (CD154; gp39) is a type II membrane protein of the TNF family (1, 2). CD40L is expressed predominantly on activated CD4+ T cells (2, 3); however, expression has also been reported on other leukocytes including CD8+ T cells (4, 5).

CD40-CD40L interactions have been shown to be a vital component of thymus-dependent (TD) humoral and cellular responses (6, 7). Studies using CD40 or CD40L knockout mice have clearly demonstrated that CD40-CD40L interactions are critical in initiating both primary and secondary TD humoral responses, in the formation of germinal centers and Ab class switching (8, 9). The abolition of TD humoral responses by disruption of CD40-CD40L interactions was shown to have resulted from both the failure to ligate CD40 on B cells and the inefficient priming of CD4+ T cells, suggesting a role for CD40-CD40L interactions in the generation of CD4+ T cell responses (10, 11).

It is now well established that efficient CD4+ T cell responses require CD40L interactions with CD40 expressed by professional APC (12–14). CD40 ligation has been shown to be critical in the development of cell-mediated autoimmune diseases such as experimental allergic encephalomyelitis (12), collagen-induced arthritis (15), and diabetes (16) as well as in other T cell-dependent responses (17, 18).

One role of the CD40-CD40L pathway appears to be the activation of professional APC (19). Ligation of CD40 on dendritic cells (DCs) has been shown to result in the prolonged survival of the DCs, the secretion of IL-12, and the up-regulation of important costimulatory molecules such as CD80, CD86, and ICAM-1 (20, 21). Recently, it has been demonstrated that such activation of DCs is required for the generation of cytotoxic CD8+ T cells and that the priming of DCs is the primary mechanism by which Ag-specific CD4+ T cells provide “help” for the initiation of Ag-specific CD8+ T cell responses (22–24).

Because CD40-CD40L interactions play a central role in the development of CD4+ and CD8+ T cell responses, the CD40L molecule makes an attractive target for therapeutic intervention to prevent T cell responses against foreign organ grafts in transplantation. Larsen et al. (25) demonstrated that the administration of an anti-CD40L mAb (MR1) around the time of transplantation led to the prolonged survival of murine cardiac allografts. Furthermore, combining CD40L blockade with CTLA4-Ig has been shown to induce long-term survival of allografts (25–27). Combining donor Ag pretreatment with CD40L blockade has also been shown to be an effective strategy to induce long-term acceptance of foreign organ grafts (28–31). Although blockade of the CD40-CD40L pathway can result in the long-term acceptance of allograft tolerance to donor, alloantigens frequently require additional therapies, and allografts can show signs of vascular damage (32).

In this study, we demonstrate that 1) donor APC do not need to be activated via the CD40-CD40L pathway to induce a CD8+ T cell response to donor alloantigen after transplantation and 2) alloantigen-specific CD8+ T cell responses are not dependent on costimulation via CD40L-CD40 interactions. We also provide indirect evidence that anti-CD40L mAb prevents CD4+ T cell-mediated rejection primarily by blocking the CD40-CD40L costimulatory interaction and not by preventing the activation of donor APC.

Materials and Methods

Animals

CBA/Ca (H2a), C57BL/10 (H2d) and NZW (H2b), B10.S (H2b) and B10.BR (H2d), BM3 (H2k, BM3.3 TCR transgenic), and DES (H2b, DES TCR transgenic) mice were housed in the part-barrier facilities of the Biomedical Services Unit, John Radcliffe Hospital.
mAbs and hybridomas

Anti-CD4 (YT3A.1), anti-CD8 (YTS169; hybridomas kindly provided by Professor H. Waldmann), and anti-CD40L (MR1) mAbs (hybridoma kindly provided by Drs. C. P. Larsen and T. C. Pearson, Emory University, Atlanta, GA) were purified and dialysed into PBS before being used in vivo. Anti-CD8-APC (53-6.7), anti-clonotypic TCR-biotin (T198 or De- sere; hybridoma kindly provided by Dr. A. L. Mellor, IMMG, Augusta, GA), anti-CD25-PE (PC61), anti-CD44-PE (IM7), anti-CD45RB-PE (16A), anti-CD62L-PE (MEL-14), and anti-CD69-PE (H1.2F3) mAbs were used for FACS analysis. For intracellular cytokine staining, anti-IL-2-PE (JE5-5H4), anti-IFN-γ-PE (XMG1.2), anti-IL-4-PE (11B11), and anti-IL-10-PE (JE55-16E3) mAbs were used. Isotype-matched mAbs R3-34-PE (rat Ig1G) and R35-38-PE (rat Ig2G2b) were used as controls. All mAbs were obtained from PharMingen/Becton Dickinson (Oxford, U.K.) unless stated otherwise.

Thymectomy and heterotopic heart transplantation

These procedures were performed as detailed previously (33).

Cell purification and CFSE labeling

CD8\(^+\) T cells from DES or BM3 TCR-transgenic mice were sorted by positive selection using the magnetic cell separation system (Miltenyi Biotec, Berisch Gladbach, Germany) (33), typically to >95% purity. More than 95% of the BM3 CD8\(^+\) T cells expressed the BM3 transgenic TCR (tg-TCR) and >80% of the DES CD8\(^+\) T cells expressed the DES tg-TCR. The isolated cells were stained with 10 \(\mu M\) 5-(and-6)-carboxyfluorescein diacetate succinimidyl ester (CFSE; Molecular Probes, Leiden, The Netherlands) before i.v. injection.

Flow cytometric analysis

Leukocytes were first stained with anti-CD8-APC and tg-TCR-biotin mAbs. The biotin-labeled mAbs were developed with a streptavidin-conjugated fluorochrome (Streptavidin-Cy-Chrome; PharMingen). Finally, the samples were stained with PE conjugated mAbs for detection of activated T cells. The samples were then fixed with 250 \(\mu L\) PBS with 2% v/v paraformaldehyde before being acquired on a FACSort (Becton Dickinson) and analyzed using the CellQuest software package (Becton Dickinson).

Intracellular cytokine staining

Spleen cells were stimulated with PMA (50 ng/ml) plus ionomycin (500 ng/ml) for 4 h at 37°C with brefeldin A (10 \(\mu g/ml\)) added for the last 2 h. Surface staining using anti-CD8-APC and tg-TCR-biotin mAbs was performed in PBS/FCS/azide + brefeldin A for 30 min. Biotin-labeled mAbs were developed with streptavidin-Cy-Chrome. The cells were then washed, permeabilized for 10 min in permeabilization buffer (PBS, 1% FCS, 0.5% saponin; Sigma, St. Louis, MO), and incubated with anti-IL-2 (2.5 \(\mu g/ml\)), anti-IFN-γ (5.0 \(\mu g/ml\)), anti-IL-4 (5.0 \(\mu g/ml\)), and anti-IL-10 (5.0 \(\mu g/ml\)) mAbs or with an isotype control mAb. All anti-cytokine Abs were PE conjugated. Samples were analyzed as previously stated.

Competitive RT-PCR

Heart grafts were removed 7 days after transplantation and were snap frozen, and total RNA was isolated using RNAzol B (Biogenesis, Bournemouth, U.K.). RNA was subjected to cDNA synthesis. The multiple competitive construct and oligonucleotide primers have been described by Reiner et al. (34). Experimental cDNA were first amplified with hypoxanthine-guanine phosphoribosyltransferase (HPRT)-specific oligonucleo- tides in the presence of equivalent amounts of the competitor construct. After electrophoresis on a 2% ethidium bromide-stained agarose gel, the bands derived from the competitor construct and the wild-type cDNA were scanned using Alphalmager software (Alpha Innotec, San Leandro, CA). The ratio of the competitor construct to wild-type cDNA allowed standardization of HPRT levels for all experimental cDNAs by adjusting the amplified amount of cDNA amplified. For measuring cytokine gene expression, equivalent amounts of the standardized cDNA were coamplified with a suitable fixed amount of the competitor construct. Again the ratio of competitor construct to cytokine-derived band was determined. All reactions were performed in triplicate, and the mean was used for further calculations. To account for minor variations in the HPRT level in the experimental samples, the final result is given as the ratio of (cytokine/competi- tor)/HPRT/competitor) in femtograms (fg) of the amount of competitor used for the amplification of the gene of interest.

Results

Anti-CD40L mAb therapy prevents CD4\(^+\) T cell-mediated rejection. All CBA/Ca mice received a C57BL/10 (B10) cardiac allograft. One group was treated with three doses of 250 \(\mu g\) MR1 mAb (given i.p.) on the day of transplant and 2 and 4 days after transplant (Fig. 1, inset). The other group did not receive any treatment ( ). Inset. Survival of a second cardiac allograft of either donor (B10; or third-party (NZW; ) origin into mice that have accepted a B10 primary cardiac graft for over 100 days after treatment with MR1.

FIGURE 1. Anti-CD40L mAb therapy prevents CD4\(^+\) T cell-mediated rejection. All CBA/Ca mice received a C57BL/10 (B10) cardiac allograft. One group was treated with three doses of 250 \(\mu g\) MR1 mAb (given i.p.) on the day of transplant and 2 and 4 days after transplantation of a MHC-mismatched (B10) cardiac allograft. All cardiac allografts were accepted indefinitely (median survival time (MST), >100 days; \(n = 11\); Fig. 1). In contrast, naive CBA/Ca recipients acutely rejected B10 grafts (MST, 10 days; \(n = 5\)).

Mice that had accepted their primary B10 grafts were tested for donor-specific transplantation tolerance. Such mice accepted a second donor-type B10 graft indefinitely (MST, >100 days; \(n = 6\); Fig. 1, inset) but rejected third-party NZW grafts promptly (MST, 18 days; \(n = 5\)).

Anti-CD40L mAb therapy does not prevent CD8\(^+\) T cell-mediated rejection of cardiac allografts

B10.BR (H2\(^b\)) mice were treated with three doses of 250 \(\mu g\) anti-CD40L mAb (MR1; given i.p.) on the day of transplant and 2 and 4 days after transplantation of a MHC-mismatched B10.S (H2\(^b\)) cardiac allograft. Surprisingly, the B10.S cardiac allografts were rejected (MST, 20 days; \(n = 4\); Fig. 2) at a rate similar to that in untreated naive B10.BR recipients. This was not because of rapid clearance of MR1 because mice that received both the initial MR1 doses and weekly administration of MR1 also rejected B10.S cardiac allograft (MST, 32 days; \(n = 5\); Fig. 2). We had previously demonstrated that both CD4\(^+\) and CD8\(^+\) T cells were able to reject a B10.S cardiac allograft independently of one another. This raised the possibility that CD8\(^+\) T cells in this strain combination had responded to and rejected the graft despite CD40L blockade. To address this question, B10.BR recipient mice were given two doses of a depleting anti-CD8 mAb 9 and 10 days before transplantation to clear peripheral CD8\(^+\) T cells. MR1 mAb therapy at the time of transplantation in these mice resulted in the prolonged survival of B10.S cardiac allografts; 60% of the grafts were accepted for over 100 days ( \(n = 5\); Fig. 2). In contrast, mice that received only the depleting anti-CD8 mAb rejected their grafts at control rates (MST, 10 days; \(n = 5\)).
We also assessed whether blockade of the CD40-CD40L co-stimulatory pathway had any effect on the ability of CD8\(^+\) T cells to proliferate and to produce inflammatory cytokines in vitro. We found that even addition of 10 \(\mu\)g/ml of MR1 mAb to MLR cultures failed to diminish the proliferative response of either purified BM3 or DES CD8\(^+\) T cells to B10 stimulators, although on day 4 of culture, production of IFN-\(\gamma\) was decreased (data not shown). However, it should be noted that even in cultures that showed a diminution of IFN-\(\gamma\) production, the amount of IFN-\(\gamma\) produced was still in excess of 6000 pg/ml.

**CD8\(^+\) T cells become activated, proliferate, and home to cardiac allografts in vivo despite CD40L blockade**

To determine whether blockade of CD40L interactions had any influence on the activation, proliferation, and homing of H2K\(^b\)-specific CD8\(^+\) T cells in response to H2K\(^b\) cardiac allografts, we used an adoptive transfer (AT) system that enabled us to visualize the behavior of T cells in vivo (33).

CBA/Ca mice were thymectomized and treated with depleting anti-CD4 (YTA3.1; 200 \(\mu\)g) and anti-CD8 (YTS169; 200 \(\mu\)g) mAbs. These mice were termed “immunocompromised” mice because they were severely depleted of peripheral T cells (95% T cell depletion) and were unable to reject a fully MHC-mismatched skin graft. Such mice were then used as “test tubes” and were injected with \(6 \times 10^6\) purified tg-TCR\(^+\) CD8\(^+\) CFSE-labeled T cells. In two separate experiments, the CD8\(^+\) T cells from BM3 mice (BM3 AT) or DES mice (DES AT) were purified and injected. One group was left untransplanted (AT only). Other mice received a B10 cardiac allograft either alone (B10 Heart) or in combination with three doses of 250 \(\mu\)g MR1 mAb (given i.p.) on the day of transplant and 2 and 4 days post transplantation (B10 heart + MR1). Seven days after transplantation, spleen, lymph nodes, and cardiac allografts were harvested, and leukocytes were prepared.

The absolute numbers of CD8\(^+\)tg-TCR\(^+\) T cells were equivalent in the spleens of all BM3 mice (AT only, 3.6 \(\times\) 10\(^5\); B10 heart, 5.12 \(\times\) 10\(^5\) \(\pm\) 0.5; B10 heart + MR1, 4.02 \(\pm\) 1.16) and DES mice (AT only, 3.4 \(\times\) 10\(^5\) \(\pm\) 0.6; B10 heart, 3.9 \(\times\) 10\(^5\) \(\pm\) 0.7; B10 heart + MR1, 3.5 \(\pm\) 0.9). However, the CFSE division profiles of the tg-TCR\(^+\) T cells in the spleens of these mice revealed that the H2K\(^b\) -specific T cells (from either the BM3 or DES mice) had proliferated after transplantation of a H2K\(^b\)\(^+\) cardiac allograft (Fig. 4). Furthermore, there was no significant difference in either the percentage of cells dividing in the presence of the MR1 mAb or in the number of divisions that these cells had undergone (Fig. 4). Analyses of the behavior of H2K\(^b\)-specific T cells present in lymph nodes were identical. It was also found that activation markers (CD69, CD44) were similarly modulated on the dividing H2K\(^b\)-specific T cells from mice that had received a cardiac allograft with or without concomitant MR1 mAb therapy (data not shown).

Next, we assessed whether the ability of the responding H2K\(^b\)-specific CD8\(^+\) T cells to produce cytokine had been impaired or deviated by the MR1 mAb treatment. Spleen leukocytes were stimulated in vitro with PMA and ionomycin for 4 h before analysis. Over this time, naive cells do not produce cytokine. The assay is an indicator of whether the H2K\(^b\)-specific CD8\(^+\) T cells had been primed to produce cytokine and of the type of cytokine profile (TC1 or TC2). However, it should be noted that this experiment does not determine whether the cells were actively producing cytokine in vivo.

There was only a small increase in the potential of BM3 tg-TCR\(^+\) T cells to produce IL-2 (Fig. 5) 7 days after transplantation of an H2K\(^b\)\(^+\) cardiac allograft, whereas many more cells were able...
to produce IFN-γ. Importantly, no differences were detected after treatment with anti-CD40L mAb. In both sets of mice the ability to produce IFN-γ was increased as a function of cell division, with most cells that had divided ≥7 times able to produce cytokine. IL-4- or IL-10-producing cells were not detected in any of the groups (data not shown). Analysis of H2Kb-specific cells from the DES AT system yielded similar results.

Graft-infiltrating cells (GICs) were prepared 7 days after transplantation. Two GIC preparations were analyzed per group. There was no significant difference in the number of H2Kb-specific T cells that had infiltrated the cardiac grafts between mice that had received MR1 mAb and those that had not (Table I). Ninety-seven percent of the GIC H2Kb-specific T cells had divided at least once (with most cells dividing at least six times; data not shown) in all groups. A similar phenotype was also observed in all groups; most cells were blasted (forward light scatterhigh), had up-regulated CD69 and CD44, and had down-regulated CD45RB and CD62L.

A proportion of cells also expressed the CD25 molecule (IL-2Rα-chain; Table I).

Hearts were harvested 7 days posttransplant from BM3 and DES AT mice that had received a B10 graft only or that had received a B10 graft and MR1 therapy. Cytokine mRNA levels were measured for IL-2, IFN-γ, IL-12, IL-4, IL-10, and inducible NO synthase (iNOS) using quantitative competitive RT-PCR. We found that in the cardiac allografts of mice that had not received MR1 therapy, there were predominant levels of the Th1 cytokines IL-2, IFN-γ, and IL-12 (Fig. 6) as well as IL-10, but there was little IL-4 mRNA. iNOS mRNA was also detected. A comparison of the mRNA levels in cardiac allografts from untreated mice to those mice that had received MR1 therapy revealed no significant increase or decrease in any of the cytokine mRNA studied (Fig. 6), suggesting that CD8+ T cells that had homed to the graft despite CD40L blockade had differentiated into Th1-like effector cells. FACS and tissue histology revealed a comparable infiltrate of
macrophages in the cardiac allografts (data not shown), and the presence of iNOS transcripts suggested that they were activated to similar extents (Fig. 6).

Discussion

The pivotal dependence of CD40-CD40L interactions in the generation of both TD humoral and cellular responses suggests that blockade of the CD40L molecule may be an effective way of preventing unwanted T cell responses to foreign organ grafts in transplantation and to self-proteins in autoimmune diseases. However, the cell types that anti-CD40L mAb affects and its mode of action remain to be clearly defined.

In the present study using both nontransgenic and transgenic mice we have demonstrated that blockade of the CD40-CD40L interaction fails to prevent CD8$^+$ T cell-mediated rejection of MHC-mismatched cardiac allografts (Figs. 1–3). Previous reports have also demonstrated that when a CD8$^+$ T cell-depleting agent (either anti-CD8 mAb or anti-asialo GM1 mAb) is combined with CD40L blockade, long-term survival or tolerance to allografts can be achieved (35, 36), suggesting that anti-CD40L mAb therapy fails to prevent CD8$^+$ T cell-mediated alloantigen rejection. However, these studies used skin grafts that are extremely sensitive to rejection. So, whether CD40L blockade has any effect on CD8$^+$ T cells has not been directly addressed. It is possible in both of these studies that the CD8$^+$ T cell response to the allograft was markedly affected by CD40L blockade but that even a suboptimal response was enough to cause rejection of a skin allograft. Alternatively, interrupting CD40L-CD40 interactions may have led to a deviated response (perhaps to a TC2 response) that still allowed graft rejection but via a different mechanism than is the case when the CD40L-CD40 pathway is intact.

To allow direct analysis of the effect of CD40-CD40L blockade on alloantigen-specific CD8$^+$ T cell responses, we used two anti-H2K$^b$ TCR transgenic mice (BM3 and DES). Although both the BM3 and DES mice are specific for the H2K$^b$ alloantigen, it has been previously shown that they differ in their activation requirements. Hua et al. (37) demonstrated that BM3 CD8$^+$ T cells can be activated without the binding of CD8, whereas DES CD8$^+$ T cells absolutely require CD8 ligation for responses to H2K$^b$. Therefore, these two populations can be considered as being representative of CD8$^+$ T cells with different affinities for alloantigen present in the normal repertoire.

To address whether blocking CD40-CD40L interactions had any effect on alloantigen-specific CD8$^+$ T cell responses directly, we analyzed alloantigen-driven responses in vitro and in vivo. Anti-CD40L mAb was found to have little effect on CD8$^+$ T cell proliferation or cytokine production in vitro (data not shown); similar findings have been reported by Larsen et al. (32). Moreover, in

Table I. Homing and phenotype of graft infiltrating CD8$^+$ T cells following transplantation$^a$

<table>
<thead>
<tr>
<th></th>
<th>BM3 AT</th>
<th>DES AT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>−MR1</td>
<td>+MR1</td>
</tr>
<tr>
<td>Number ($10^6$)</td>
<td>39 ± 11</td>
<td>51 ± 3</td>
</tr>
<tr>
<td>% Divided</td>
<td>97.7 ± 0.6</td>
<td>97.7 ± 0.7</td>
</tr>
<tr>
<td>% FSC$^{high}$</td>
<td>64.3 ± 1.6</td>
<td>64.7 ± 0.1</td>
</tr>
<tr>
<td>% CD25$^{high}$</td>
<td>13.3 ± 1.3</td>
<td>12.1 ± 1.7</td>
</tr>
<tr>
<td>% CD69$^{high}$</td>
<td>64.3 ± 5.5</td>
<td>65.7 ± 1.2</td>
</tr>
<tr>
<td>% CD44$^{low}$</td>
<td>98.1 ± 0.3</td>
<td>98 ± 0.1</td>
</tr>
<tr>
<td>% CD45RB$^{low}$</td>
<td>76.6 ± 2.9</td>
<td>75.2 ± 4.0</td>
</tr>
<tr>
<td>% CD62L$^{low}$</td>
<td>84 ± 3.4</td>
<td>85.9 ± 3.7</td>
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$^a$ Cardiac allografts from the groups described in Fig. 5 were harvested 7 days after transplantation, and the graft-infiltrating cells (GICs) were prepared. Using four-color flow cytometry allowed the evaluation of the number of CD8$^+$ T cells (GICs) within each cardiac allograft as well as their division history and phenotype. Results are expressed as the mean of two GIC preparations per group ± SD.

FIGURE 6. Expression of cytokines within cardiac allografts is unchanged by anti-CD40L mAb therapy. Hearts were harvested 7 days posttransplant from BM3 and DES AT mice that had received a B10 graft only or that had received a B10 graft and MR1 therapy. Cytokine mRNA levels were measured for IL-2, IFN-γ, IL-4, IL-10, and iNOS using quantitative competitive RT-PCR. All analyses contained three to four heart samples from different mice. Results are expressed as the mean ratio of (cytokine/competitor)/(HPRT/competitor) in femtograms (fg) ± SD.
vivo in two distinct models, we found that anti-CD40L mAb administration had no effect on CD8+ T cell proliferation (Fig. 4), activation, or differentiation after transplantation (Fig. 5). Furthermore, CD40L blockade did not alter the homing of the activated cells to the graft (Table I) or the production of Th1 effector cytokine mRNA within the graft itself (Fig. 6).

Many CD8+ T cell responses are dependent on CD4+ T cell help. It has been demonstrated that T cell help is mediated by the activation of APC by CD40 interactions with CD40L expressed by activated CD4+ T cells (22-24). However, DCs can be activated without CD40-CD40L interactions, for example, by viral infection (22). This is a likely explanation of why disruption of the CD40-CD40L interaction fails to prevent cytotoxic T lymphocyte priming and/or cell-mediated immunity in some models of viral or bacterial infections (38, 39).

The present study shows that after transplantation, CD8+ T cells can respond to donor APC whether the CD40-CD40L pathway is blocked or not. Therefore, we propose that the nonspecific inflammation associated with transplantation can activate or prime donor DCs resident in the transplanted tissue to stimulate alloantigen-specific CD8+ T cells after they migrate to the draining lymphoid organ. Recently, it has been found that DCs can be activated both by inflammation and by stressed/necrotic cells (40). Given the nonspecific inflammation and hypoxia associated with transplantation, activation of resident DC might be expected and would be consistent with the observations presented in this study. Interestingly, a recent study showed that when DES cells were transferred to a syngeneic recipient that was subsequently challenged with a donor-specific transfusion (DST) with or without MR1, the DES cells made an abbreviated response in the presence of MR1 and underwent deletion (41). In this system because of the use of a DST (presumably containing APC that are not activated), the response of the DES cells is likely to be dependent on priming of the donor APC via CD40-CD40L interactions by CD4+ T cells. Therefore, using a DST instead of an allograft as the form of initial alloantigen challenge converted the DES response from being independent of CD40-CD40L interactions (presented in this report) to one that is dependent. These data are consistent with donor APC being activated by transplantation and suggest that alloantigen pretreatment at the time of CD40L blockade may be of greater benefit than at the time of transplantation. This question is currently being addressed in our laboratory.

Buhllmann et al. (42) have also demonstrated in a model of graft-versus-host disease that an effective CD8+ T cell response required CD40-CD40L interactions, in contrast to the present report. However, as with the previous study, alloantigen is likely to be encountered on APC that are not activated after transfer of T cells into a naive unmanipulated host. Therefore, the activation status of the donor APC seems to dictate whether the CD8+ T cell response to alloantigen is dependent on CD40-CD40L interactions.

Whether donor passenger DCs stimulate a response resulting in CD8+ T cell-mediated rejection of a transplanted organ is likely to depend on the precursor frequency of donor-reactive CD8+ T cells in the recipient. Different donor-recipient combinations showed differing involvement of CD4+ and CD8+ T cells in graft rejection (43). In this study, the B10.S to B10.BR strain combination exemplifies a combination in which both CD4+ and CD8+ T cells can initiate graft rejection independently of one another. However, in the B10 to CBA strain combination, we have previously demonstrated that removal or tolerization of CD4+ T cells can initiate graft rejection independently of one another. However, in the B10 to CBA strain combination, we have previously demonstrated that removal or tolerization of CD4+ T cells results in the failure of CD8+ T cells to reject a cardiac allograft (44). This suggests that either the donor DCs in this strain combination are not primed to activate alloantigen-specific CD8+ T cells after transplantation or that the precursor frequency of responding CD8+ T cells is too low to acutely reject a cardiac allograft. We would favor the latter explanation because B10 cardiac grafts efficiently stimulated BM3 and DES CD8+ T cells in the AT system in the absence of CD4+ T cells. Also, the response of BM3 cells was unaltered by MR1 when injected into a naive CBA recipient (precursor frequency was increased because of Ig-TCR+ cells and endogenous repertoire) or when 2 × 106 BM3 cells were injected into an “empty” CBA (precursor frequency decreased compared with the present study; data not shown).

The response of alloantigen-specific T cells in the AT system despite CD4+ T cell depletion and CD40L blockade also suggests that the CD40-CD40L interaction is not an important costimulatory pathway for CD8+ T cells. Perhaps this is not surprising given the restricted expression of CD40L on CD8+ T cells (4, 5). However, other members of the TNF family have now been identified as important costimulatory molecules such as OX40 (45), TNF-related activation-induced cytokine (46), and 4-1BB (47). Therefore, CD8+ T cells may utilize other TNF-related proteins in addition to CD28 (48), ICOS (49), and ICAM-1 (50, 51) molecules as costimulatory molecules in the absence of CD40L costimulation, although the dependence of CD8+ T cell responses on the CD28-B7 costimulation pathway has also recently been questioned (52).

Taken together, the present study suggests that blockade of costimulatory molecules utilized by CD8+ T cells, in addition to anti-CD40L mAb therapy, would be extremely beneficial in inducing long-term allograft acceptance in transplantation. Indeed, combining CTLA4 Ig with CD40L blockade in experimental transplantation has proved far more successful at inducing long-term graft survival than CD40L blockade alone (26, 32).

Recently, Kirk et al. (27) have shown that in a preclinical model of kidney transplantation, prolonged administration of an extended course of anti-CD40L mAb resulted in long-term graft acceptance without the vascular changes associated with chronic rejection. In this model no additional therapy was given. This raises the question of why graft-reactive CD8+ T cells failed to mediate any damage to the kidney grafts in this study.

Although anti-CD40L does not affect CD8+ T cell responses directly after transplantation, CD8+ T cell responses could be altered indirectly by CD4+ T cells. One proposed result of anti-CD40L blockade around the time of transplantation is the preferential development of Th2 T cells (29, 31). Given the mutually suppressive relationship between Th2 and Th1 CD4+ T cells (53), enforced Th2 cytokine expression by CD4+ T cells responding to alloantigen during CD40L blockade may affect CD8+ Th1 effector and cytotoxic T cell development, thus potentially limiting the ability of CD8+ T cells to affect graft destruction. However, when we studied the response of CD8+ T cells to heart allografts in the presence of CD4+ T cells during CD40L blockade, the response was identical with that in which the CD40L-CD40 pathway was intact (data not shown).

Another possibility is that donor-derived APC that do not require activation via CD40-CD40L interactions to elicit a CD8+ T cell response are unique to heart transplantation. The possibility exists that different tissues contain different APC and undergo different nonspecific inflammatory events after transplantation. So, for example, kidney-derived donor APC after transplantation may not be primed to activate donor-reactive CD8+ T cells in the absence of CD40-CD40L interactions. Further study is required to determine whether the type of graft determines the effectiveness of anti-CD40L mAb therapy at controlling CD8+ T cell responses.

The demonstration that, after heart transplantation, donor APC can stimulate CD8+ T cells without first being primed suggests that these donor APC express elevated levels of costimulatory...
molecules. However, despite the donor APC being primed, administration of the anti-CD40L mAb was still able to prevent CD4+ T cell-mediated rejection in two different strain combinations (Figs. 1 and 2). These data suggest that the mode of action of the anti-CD40L mAb is to prevent CD40-CD40L interactions from costimulating the response of CD4+ T cells, but not by preventing priming of donor APC, as recently shown by Shepherd and Kerkvliet (54). The result of recognition of alloantigen by CD4+ T cells in the absence of CD40L costimulation is currently unknown, but immune deviation to a Th2 response (29, 31) and dominant-negative signals mediated through CTLA4 (31) resulting in premature deletion (55, 56) have been proposed as possible outcomes.

In conclusion, we have shown that CD40-CD40L interactions are not required for donor APC activation after transplantation and that CD8+ T cell responses are not dependent on CD40-CD40L costimulation. These data suggest that although anti-CD40L mAb therapy is effective at blocking CD4+ T cell-mediated rejection, the inclusion of reagents directed at CD8+ T cells will be a worthwhile addition to any potential clinical therapy.

Acknowledgments

We thank Dr. Andrew L. Mellor for the generous gifts of the BM3 and DES mice used in this study as well as for the anti-clonotype mAbs and Drs. S. Reiner and S. Miller for kindly providing the multiple competitive construct. We also thank Dr. Andrew R. Bushell for critical review of the manuscript and Clare Smith, Stuart E. Turvey, and Marco-Antonio Reis e Moura for technical assistance.

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References


11. van Essen, D., H. Kikutani, and D. Gray. 1995. CD40 ligand-transduced costimula-


and prolonged graft survival in a CTLA4-dependent manner. J. Immunol. 164: 512.


