CD40 Signaling Replaces CD4+ Lymphocytes and Its Blocking Prevents Chronic Rejection of Heart Transplants

Michael P. Fischbein, Abbas Ardehali, James Yun, Stephen Schoenberger, Hillel Laks, Yoshihito Irie, Paul Dempsey, Genhong Cheng, Michael C. Fishbein and Benjamin Bonavida

J Immunol 2000; 165:7316-7322; doi: 10.4049/jimmunol.165.12.7316
http://www.jimmunol.org/content/165/12/7316

This article cites 43 articles, 14 of which you can access for free at: http://www.jimmunol.org/content/165/12/7316.full#ref-list-1

Why The JI? Submit online.
- Rapid Reviews! 30 days* from submission to initial decision
- No Triage! Every submission reviewed by practicing scientists
- Fast Publication! 4 weeks from acceptance to publication

*average

Subscription Information about subscribing to The Journal of Immunology is online at: http://jimmunol.org/subscription

Permissions Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

Email Alerts Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
CD40 Signaling Replaces CD4+ Lymphocytes and Its Blocking Prevents Chronic Rejection of Heart Transplants

Michael P. Fischbein,*† Abbas Ardehali, † James Yun, ‡‡ Stephen Schoenberger, § Hillel Laks, Yoshimoto Irie, † Paul Dempsey,* Genhong Cheng,* Michael C. Fishbein, † and Benjamin Bonavida 1*

Chronic rejection remains the major obstacle to long term survival in heart transplant recipients. The cellular and molecular mechanisms that underlie chronic rejection are not known, and their discovery can form the basis of clinical intervention. Several investigators have suggested that the development of chronic rejection in solid organ transplants is dependent on help mediated by CD4+ lymphocytes. Importantly, the mechanism through which help is provided has not been fully delineated in transplant rejection. Using a murine heterotopic heart transplant model without immunosuppression, this study defines the functional role of CD4+ lymphocytes in chronic rejection. In an MHC class II-mismatched model, we demonstrate that chronic rejection was absolutely contingent on the presence of CD4+ lymphocytes. Importantly, here we report that signaling through CD40 can replace the requirement of CD4+ lymphocytes, demonstrated by the development of chronic rejection in CD4 knockout recipients treated with a CD40-activating mAb (FGK45). The return of rejection appears to be a CD8+ lymphocyte-dependent process, not by the absence of rejection in FGK45-treated recombinase-activated gene knockout (CD4+ and CD8+ lymphocyte-deficient) recipients. The CD40 signaling pathway works independently of B7-CD28 costimulation, as indicated by the development of severe chronic rejection in CD28 knockout recipients. Importantly, this study provides evidence that CD40 ligand-targeted therapies may prevent chronic rejection only in strain combinations where CD4+ lymphocyte help is absolutely required. The Journal of Immunology, 2000, 165: 7316–7322.

Heart transplantation has become an accepted treatment option for patients with end-stage heart disease. However, cardiac allograft vasculopathy (CAV), or chronic rejection, remains the major cause of death after the first post-transplantation year (1). CAV is characterized by a diffuse intimal proliferation in the blood vessels supplying the heart, leading to luminal obliteration and subsequent graft failure. Although significant insights have been made in allograft rejection, the molecular mechanisms behind the genesis of CAV development are not known.

Activated T lymphocytes are known to play a central role in the rejection of solid organ transplants (2–5). We have recently reported an MHC class II-mismatched murine model of CAV in which chronic rejection reproducibly developed by 24 days post-transplantation. Using both lymphocyte knockout recipients as well as adoptive transfer studies we have demonstrated that CD4+ lymphocytes were absolutely required for CAV development in this model system (44). However, the role of the CD4+ lymphocytes in mediating CAV in this model has not been fully elucidated. Among their many functions, CD4+ lymphocytes are known to provide help for effector cells such as CTL, B lymphocytes, and macrophages, classically via the secretion of cytokines. Alternatively, help may be delivered through a reciprocal dialogue between CD4+ lymphocytes and APCs via the CD40 ligand (CD40L)-CD40 signaling pathway (6,7).

CD40L is a member of the TNF gene family, and is expressed primarily on activated CD4+ lymphocytes. The counter-receptor for CD40L is CD40, a member of the TNF receptor superfamily, found on APCs, including dendritic cells, macrophages, and B cells (8–12). Although the mechanism seems to depend on the experimental model system and strain combination studied, possible outcomes of CD40L-CD40 engagement include the direct activation of CD4+ lymphocytes via CD40L and the activation of APCs via CD40 (9–12).

Recent data have implicated a role for CD40L-CD40 signaling in acute and chronic rejection transplantation models. Kirk et al. have recently demonstrated that mAb against CD40L prevents acute renal allograft rejection in non-human primates (13). Furthermore, Larsen et al. illustrated that the simultaneous blockade of CD28 and CD40L costimulatory pathways prevents chronic rejection in murine cardiac allografts (14). The aim of the current study is to define the role of CD40L-CD40 signaling in a murine model of CAV without immunosuppression. The hypothesis we wished to test is whether CD40L-CD40L interactions are critical for CD4+ lymphocytes to mediate the development of CAV. Identification of such an essential receptor-ligand pair in CAV would provide a theoretical framework for the development of targeted therapeutics aimed at increasing graft function and acceptance.

Materials and Methods

Animals

B6.C-H2<sup>+</sup>12, C57BL/6 (wild-type, CD4<sup>+/−</sup> knockout, CD8<sup>+/−</sup> knockout, nude, CD40L<sup>+/−</sup> knockout, CD28<sup>+/−</sup> knockout, recombinase-activating gene).
gene (RAG-1−/− knockout, and µMT), B10.A, and B10.BR strains of female mice, 7–11 wk old, were purchased from The Jackson Laboratory (Bar Harbor, ME) and housed under sterile conditions. They were fed autoclaved rodent Chow (Purina Mills, St. Louis, MO) and water ad libitum. The B6.C-H2m12 and C57BL/6 strains of mice differ in one locus of MHC class II Ag, but are matched at all other major and minor histocompatibility Ags. All animals received humane care in compliance with the Principles of Laboratory Animal Care and the Guide for the Care and Use of Laboratory Animals prepared by the Institute for Laboratory Animal Resources and published by the National Institutes of Health.

Transplantation

The intra-abdominal heterotopic heart transplantation was performed according to the technique previously described by Corry et al. (15). Briefly, through a midline abdominal incision, the aorta of the donor heart was anastomosed to the recipient’s infrarenal abdominal aorta, and the pulmonary artery of the donor heart was anastomosed to the inferior vena cava with 10-0 nylon suture. The donor ischemia time was approximately 45 min. Function of the allografts was assessed through abdominal palpation and scored on a scale of 0–4 (0 indicates absence of contractions, and 4 indicates normal beating).

Experimental groups

B6.C-H2m12 mouse hearts were transplanted into the following C57BL/6 recipients: 1) wild-type control (n = 6), 2) CD4−/− knockout (n = 6), 3) CD8−/− knockout (n = 6), 4) CD40L−/− knockout (n = 6), 5) wild-type control and MR-1 (CD40L blocking; n = 6), 6) CD4−/− knockout and FGK45 (CD40 activating Ab; n = 5), 7) CD4−/− knockout and rat IgG isotype control Ab (n = 3), 8) RAG-1−/− knockout and CD40L-CD40 blockade prevents CAV

Results

CD40L-CD40 blockade prevents CAV

To test whether CD4+ lymphocytes mediate the development of CAV by triggering the CD40 pathway, donor hearts were transplanted into CD40L-deficient recipients. In contrast to the intimal lesions detected in the donor hearts transplanted into C57BL/6 recipients (wild-type control), the donor hearts in CD40L−/− knockout recipients failed to develop chronic rejection (50% ± 7 vs 0 ± 0%; p < 0.05; Fig. 1A, 1B, and Table I). Notably, treatment of C57BL/6 wild-type recipients with the anti-CD40L-blocking Ab MR-1 also prevented lesion development (0 ± 0%; Fig. 1C and Table I).

Histologically, there was almost complete absence of CD4+ lymphocytes, CD8+ lymphocytes, and monocytes/macrophages in allo- grafts from both CD40L−/− knockout and MR-1-treated wild-type recipients (Fig. 2, A and B, and Table II). In contrast, the intimal lesions in wild-type recipients were predominately composed of CD4+/CD8− lymphocytes, macrophages, and smooth muscle cells, deposited in a substance rich in extracellular matrix. In addition, hearts transplanted into CD40L−/− knockout and MR-1-treated groups showed a significant decrease in ICAM-1 and VCAM-1 expression compared with the hearts transplanted into wild-type recipients (data not shown). These experiments indicate that CD40L-CD40 signaling plays an important role in mononuclear cell recruitment, adhesion molecule up-regulation, and CAV development.

Activation of CD40 with mAb restores CAV development in CD4+ lymphocyte-deficient recipients

Since CD4+ lymphocytes activate APCs via CD40L-CD40 signaling, we hypothesized that artificial activation of CD40 (via a CD40-activating Ab) could replace the function of CD4+ lymphocytes and induce CAV development in CD4-deficient recipients. Donor hearts were transplanted into CD4−/− knockout mice and administered either the CD40 activating Ab, FGK45, or an isotype control Ab. In the absence of recipient CD4+ lymphocytes, chronic rejection was restored in part by the activation of CD40 via FGK45 (22 ± 1%; Fig. 1D and Table I). In contrast, the hearts in isotype control Ab-treated recipients did not develop chronic rejection (0 ± 0%). Although CAV developed in the hearts transplanted into the FGK45-treated recipients, lesion severity was significantly reduced compared with the donor hearts transplanted into wild-type recipients (22 ± 1 vs 50 ± 7%; p < 0.05, respectively). Mononuclear cells were absent in the donor hearts transplanted into CD4−/− knockout recipients treated with isotype control Ab. However, both graft-infiltrating CD8+ lymphocytes and monocytes/macrophages were detected immunohistochemically in the donor hearts following treatment with FGK45 (Fig. 2, C and D, and Table II).
Finally, isografts (C57BL/6 donors to C57BL/6 recipients) were administered FGK45 and sacrificed at 24 days. Donor hearts in isograft controls did not develop lesions (0%–6%). These experiments demonstrate that activation of CD40 can substitute for the requirement of CD4+ lymphocytes and partially restore CAV development, and induction of CAV development in FGK45-treated groups is an alloantigen-dependent event.

Because the donor hearts transplanted into FGK45-treated CD4−/− knockout recipients developed intimal lesions, we reasoned that allorecognition was occurring via CD8+ lymphocytes. Thus, donor hearts were transplanted into RAG−/− knockout recipients and CD40-activating mAb (FGK45) developed less severe lesions compared with the donor hearts transplanted into wild-type recipients.

CAV development is absent in RAG−/− knockout recipients treated with mAb-mediated CD40 activation

### Table 1. Intimal proliferation in donor hearts

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Donor</th>
<th>Recipient</th>
<th>Intimal Thickening (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>BM12</td>
<td>C57BL/6</td>
<td>50.0 ± 7.0</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>BM12</td>
<td>CD4−/−</td>
<td>19.0 ± 6.0*</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>BM12</td>
<td>CD8−/−</td>
<td>19.0 ± 6.0</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>BM12</td>
<td>CD40L−/−</td>
<td>0.0 ± 0.0*</td>
</tr>
<tr>
<td>V</td>
<td>6</td>
<td>BM12</td>
<td>C57BL/6 + MR-1</td>
<td>0.0 ± 0.0*</td>
</tr>
<tr>
<td>VI</td>
<td>5</td>
<td>BM12</td>
<td>CD4−/− + FGK45</td>
<td>22.0 ± 1.0*</td>
</tr>
<tr>
<td>VII</td>
<td>3</td>
<td>BM12</td>
<td>CD4−/− + rat IgG</td>
<td>0.0 ± 0.0*</td>
</tr>
<tr>
<td>VIII</td>
<td>3</td>
<td>BM12</td>
<td>RAG−/− + FGK45</td>
<td>0.0 ± 0.0*</td>
</tr>
<tr>
<td>IX</td>
<td>6</td>
<td>BM12</td>
<td>CD28−/−</td>
<td>40.0 ± 8.0</td>
</tr>
<tr>
<td>X</td>
<td>4</td>
<td>BM12</td>
<td>μMT (B cell deficient)</td>
<td>55.0 ± 3.8</td>
</tr>
<tr>
<td>XI</td>
<td>3</td>
<td>C57BL/6</td>
<td>C57BL/6 + FGK45</td>
<td>0.0 ± 0.0*</td>
</tr>
<tr>
<td>XII</td>
<td>6</td>
<td>B10.A</td>
<td>B10.BR</td>
<td>44.0 ± 4.9</td>
</tr>
<tr>
<td>XIII</td>
<td>3</td>
<td>B10.A</td>
<td>B10.BR + MR-1</td>
<td>30.0 ± 2.1**</td>
</tr>
<tr>
<td>XIV</td>
<td>3</td>
<td>B10.A</td>
<td>B10.BR + rat IgG</td>
<td>41.2 ± 3.7</td>
</tr>
</tbody>
</table>

* p < 0.05 vs group I; ** p < 0.05 vs group XII.
recipients (CD4\(^+\) and CD8\(^+\) lymphocytes absent) and treated with FGK45. In the absence of both CD4\(^+\) and CD8\(^+\) lymphocytes, the artificial activation of CD40 did not restore the development of rejection (0\% vs 60\%). Histologically, there was complete absence of all graft-infiltrating mononuclear cells.

**CAV development is reduced in an MHC class I-mismatched model treated with CD40L-blocking mAb**

Recent studies have suggested that CD40L-CD40 blockade is insufficient in blocking alloreactive CD8\(^+\) lymphocyte function (16, 17). Hence, we hypothesize that CD40L-CD40 blockade may be effective in CAV prevention only in donor-recipient strain combinations in which CD4\(^+\) lymphocyte help is absolutely required. To test this hypothesis, we examined the effect of CD40L blockade on B10.A donor hearts transplanted into B10.BR recipients (MHC class I-disparate Ags). In this strain combination, our laboratory has previously reported that CD8\(^+\) lymphocytes participate in CAV development independently of CD4\(^+\) lymphocyte help (CD4\(^+\) lymphocytes depleted with GK1.5 mAb; see Footnote 3).

After 24 days, donor hearts transplanted into MR-1-treated B10.BR recipients developed CAV, although at a level significantly reduced from that in recipients treated with isotype control Ab (306\% vs 416\%; \(p<0.05\), respectively; Table I). However, the extent of CAV development in the donor hearts was similar in MR-1 treated B10.BR recipients and CD4\(^+\) lymphocyte-depleted B10.BR recipients (treated with GK1.5).

Histologically, there was a decrease in the number of graft-infiltrating CD8\(^+\) lymphocytes in MR-1 treated B10.BR recipients. Importantly, the number of CD8\(^+\) lymphocytes that homed to the cardiac allografts was

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Recipient</th>
<th>CD4</th>
<th>CD8</th>
<th>Mac</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>6</td>
<td>C57BL/6</td>
<td>2.0±0.0</td>
<td>1.3±0.7</td>
<td>3.6±0.4</td>
</tr>
<tr>
<td>II</td>
<td>6</td>
<td>CD4(^{-/-})</td>
<td>0.0±0.0*</td>
<td>0.3±0.2*</td>
<td>0.5±0.2*</td>
</tr>
<tr>
<td>III</td>
<td>6</td>
<td>CD40L(^{-/-})</td>
<td>0.0±0.0*</td>
<td>0.0±0.0*</td>
<td>0.5±0.2*</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>C57BL/6 + MR-1</td>
<td>0.0±0.0*</td>
<td>0.0±0.0*</td>
<td>0.5±0.1*</td>
</tr>
<tr>
<td>V</td>
<td>5</td>
<td>CD4(^{-/-}) + FGK45</td>
<td>0.0±0.0*</td>
<td>1.5±0.9</td>
<td>2.3±0.7</td>
</tr>
</tbody>
</table>

* \(p<0.05\) vs group I.
similar in MR-1-treated and CD4^- lymphocyte-depleted groups. Therefore, in an MHC class I-mismatched model of CAV (dependent on both CD4^- and CD8^- lymphocytes), CD40 ligand blockade decreases CAV development, but is unable to abrogate it.

CD40L-CD40 signaling does not mediate CAV development via the B7-CD28 pathway in this model

Several studies have illustrated that CD40 engagement leads to APC activation via the up-regulation of B7 surface proteins (18–21). Therefore, we hypothesized that CD40L-CD40 signaling in the development of chronic rejection is dependent on the B7-CD28 signaling pathway. To test this hypothesis, donor hearts were transplanted into CD28^-/- knockout recipients. After 24 days, the donor hearts in these recipients developed severe intimal lesions, similar in severity to donor hearts transplanted into wild-type control recipients (40 ± 8 vs 50 ± 7%; Table I). The intimal lesions were similar in cellular content to those noted in the hearts transplanted into wild-type recipients. Therefore, the requirement of CD40 signaling in chronic rejection is not dependent on the B7-CD28 pathway in this system.

CD40L-CD40 signaling does not induce CAV development via B cell activity

The above findings illustrated that one potential role of CD4^+ lymphocytes in the pathogenesis of CAV is to activate APCs via the CD40L-CD40 signaling pathway. However, the target APC in this model has not been delineated. Because several investigators have reported that the humoral immune system plays a role in chronic rejection (4, 22), we hypothesized that the essential helper signal is delivered via CD40L on the surface of the CD4^+ lymphocyte and through CD40 on the surface of the B cell. To test this hypothesis, donor hearts were transplanted into μMT recipients, which lack mature B cells (23). Lesions in donor hearts transplanted into μMT recipients were not significantly different from wild-type controls (55 ± 4 vs 50 ± 7%, respectively; Table I). These results suggest that CD40L-CD40-mediated B cell proliferation and Ig isotype switching are not essential mechanisms for CAV development in this strain combination.

Discussion

In this study we used a nonimmunosuppressed murine model of CAV to further understand the role of CD40L-CD40 signaling in chronic rejection of transplanted hearts. This study shows that in a donor-recipient strain combination in which CAV is dependent on the presence of CD4^+ lymphocyte help, 1) CAV development is contingent upon CD40L-CD40 engagement; 2) CD40 signaling can partially replace CD4^+ lymphocytes in the pathogenesis of CAV; and 3) CD40L-CD40 signaling in CAV acts through an alternative pathway independent of B7-CD28 costimulation.

Using an MHC class II-mismatched model, we have found that the development of chronic rejection is absolutely contingent on the helper function of CD4^+ lymphocytes. However, the mechanism through which help is delivered has not been fully elucidated. Classically, CD4^+ lymphocytes are thought to provide their help indirectly through cytokine secretion, which activates mononuclear cells, including APCs (24, 25). Alternatively CD4^+ lymphocyte help may be delivered directly by a membrane-membrane interaction, via CD40L on the CD4^+ lymphocyte and CD40 on the APC (26). While several studies have established the importance of CD40L-CD40 signaling in acute rejection/tolerance (13, 27–30), its role in the pathogenesis of chronic rejection remains unresolved.

This study provides evidence that CD40L-CD40 signaling plays a fundamental role in the development of CAV in the absence of any immunosuppression. This finding was illustrated by the lack of intimal proliferation in donor hearts transplanted into CD40L^-/- knockout recipients and confirmed by treatment of wild type recipients with anti-CD40L blocking Ab (MR-1). Several studies have also demonstrated the importance of CD40 signaling in T lymphocyte-dependent immune responses. Larsen et al. reported both the prolongation of graft survival and the prevention of chronic rejection in cardiac allografts treated with the combination therapy, CTLA-4 Ig and MR-1 (14). In marked contrast to this study, MR-1-treated allografts developed intimal lesions, whereas the simultaneous blockade of both CD28 (CTLA-4 Ig) and CD40 (MR-1) signaling pathways was required to effectively inhibit chronic rejection (14). Using a murine aortic allograft model, Subbotin et al. also reported that the blockade of both CD28 and CD40L-CD40 costimulatory pathways was required to prevent chronic rejection (31). An explanation for the requirement of both CD28/CD40L blockade may be attributed to the full Ag-mismatched strain combinations used in the previous studies. In this study B6.C-H2b^nu/2 and C57BL/6 mice differ in only one locus of MHC class II Ag (I-A). Nonetheless, in this model, CD4^- lymphocytes cannot provide the help required for CAV development in the absence of CD40L.

Signaling through CD40 can partially replace the function of CD4^+ helper cells in the pathogenesis of CAV, indicated by the rescue of intimal lesion development in donor hearts transplanted into CD4^-/- knockout recipients treated with FGK45. The return of lesions must be considered an alloantigen-dependent event, since no lesions developed in FGK45-treated isograft controls. CD40 ligation has been shown to induce increased APC activity via the up-regulation of adhesion and costimulatory molecules as well as the secretion of proinflammatory cytokines (20, 21). Therefore, a potential mechanism for the return of chronic rejection in donor hearts transplanted into FGK45-treated CD4^-/- knockout recipients is the artificial activation of APCs, which can then effectively activate CD8^- lymphocytes. Supporting this hypothesis, CD8^- lymphocytes infiltrated the allograft in FGK45-treated CD4^-/- knockout recipients, but were absent in the allografts transplanted into either CD4^-/- knockout, CD40L^-/- knockout, or MR-1-treated recipients. Furthermore, lesions did not develop in the absence of CD8^- lymphocytes, as noted in FGK-treated RAG^-/- knockout recipients (absence of both CD4^+ and CD8^- lymphocytes).

It is intriguing that CD8^- lymphocytes may induce CAV development in an MHC class II-mismatched model. We have previously reported that CD8^- lymphocytes play a role in the progression of chronic rejection in the presence of CD4^- lymphocytes in vivo. Further, we have demonstrated that MHC class II Ags can activate isolated CD8^- lymphocytes and contribute to the alloimmune response in vitro (44). Classically, CD8^- lymphocytes recognize Ags that are presented as peptide complexes with MHC class I molecules (32–34). MHC class I-restricted presentation is usually associated with degradation of endogenous proteins and is often considered inaccessible to exogenous Ags (32–34). However, there is some evidence that exogenous proteins (shed foreign MHC class II protein, in our model) can gain access to the MHC class I processing pathway, often referred to as cross-priming (32–34). While our studies did not test specifically for cross-priming, we speculate that the cross-priming phenomenon may provide a potential mechanism to explain CD8^- lymphocyte allorecognition in a non-MHC class I-mismatched model.

Importantly, the donor hearts transplanted into FGK45-treated CD4^-/- knockout recipients never developed the extent of chronic rejection noted in the donor hearts transplanted into wild-type recipients. Although an FGK45 dose-response curve was performed,
it is possible that optimal concentrations were not achieved. Alternatively, the nature of the help provided by CD4+ lymphocytes may be only partially attributed to the interaction with CD40. It is conceivable that in addition to CD40 activation, the immunoregulatory cytokines secreted by activated CD4+ lymphocytes are required to develop a strong alloimmune response. Therefore, we propose that in this model, both the classic and alternative models probably play a role in chronic rejection.

CD40 ligation has been shown to augment APC activity by up-regulating B7 and other costimulatory molecules (7, 18–21). It also induces the secretion of proinflammatory cytokines, including IL-12 secretion from APCs (18–21, 25). While several antiviral models have shown that CD40-dependent T lymphocyte activation was mediated via B7 up-regulation (18, 30, 35), the mechanism of CD40 signaling in the setting of transplantation remains controversial. Hancock et al. showed that although CD40L blockade almost abolished B7.1 expression on graft-infiltrating cells, the functional blockade of B7.1 did not mediate the same graft-prolonging effects as anti-CD40L (27). In contrast, Forster et al. suggested the importance of CD40-mediated B7 expression in generating an alloimmune response in cardiac allografts (30). We postulated that if the up-regulation of B7 was the major mechanism by which CD40 activation regulates T lymphocyte activation, then hearts transplanted into CD28+/− knockout recipients should not develop chronic rejection. In contrast to the absence of CAV in donor hearts transplanted into CD40L−/− knockout recipients, allografts in CD28−/− knockout recipients developed severe intimal lesions. Therefore, our CD28−/− knockout study complements the findings described in CD28 blocking Ab (CTLA-4 Ig) reports (27). Although this study does not identify the mechanism of CD40 signaling in allograft chronic rejection, the above experiments suggest that the CD40 signaling pathway works independently of the CD28 signaling pathway.

Ig deposition has been reported to play an important role in the initiation of both acute and chronic rejection in animal models (2, 4, 22). In addition, CD40L-CD40 interaction is essential in germinal center formation, Ig isotype switching, B cell proliferation, and memory B cell formation (20). Therefore, we postulated that CD40 signaling and the development of chronic rejection may operate through activation of CD40 on B cells. To test this theory, donor hearts were transplanted into μMT mice, which lack mature B cells (23). As noted, the development of chronic rejection in these mice was not significantly different from that in wild-type control recipients. Larsen et al. reported that B cells are not required for acute allograft rejection, as indicated by the prompt rejection of μMT-deficient mice in a fully mismatched cardiac transplant model (28, 29). Although the identity of the recipient APCs involved in CAV has not been identified, our results illustrate that mature B cells are not required in this model. Furthermore, in this model, Ig deposition was not required for the initiation of CAV. Consequently, the rescue of lesion development in mice transplanted into μMT mice independent of CD40 signaling was mediated via B7 up-regulation (18, 30, 35), the mechanism of CD40 signaling in the setting of transplantation remains controversial. Forster et al. showed that although CD40L blockade almost abolished B7.1 expression on graft-infiltrating cells, the functional blockade of B7.1 did not mediate the same graft-prolonging effects as anti-CD40L (27). In contrast, Forster et al. suggested the importance of CD40-mediated B7 expression in generating an alloimmune response in cardiac allografts (30). We postulated that if the up-regulation of B7 was the major mechanism by which CD40 activation regulates T lymphocyte activation, then hearts transplanted into CD28+/− knockout recipients should not develop chronic rejection. In contrast to the absence of CAV in donor hearts transplanted into CD40L−/− knockout recipients, allografts in CD28−/− knockout recipients developed severe intimal lesions. Therefore, our CD28−/− knockout study complements the findings described in CD28 blocking Ab (CTLA-4 Ig) reports (27). Although this study does not identify the mechanism of CD40 signaling in allograft chronic rejection, the above experiments suggest that the CD40 signaling pathway works independently of the CD28 signaling pathway.

This study suggests that CD40L-CD40 signaling plays an important role in the pathogenesis of CAV in an MHC class II-mismatched model. Notably, in the B6.C-H-2(+)12 and C57BL/6 strain combination (MHC class II-disparate Ags), CAV development is absolutely contingent on CD4+ lymphocyte help. In contrast, CD40L blockade did not completely prevent CAV development in an MHC class I-mismatched model system. Interestingly, in the B10.A and B10.BR strain combination (MHC class I-disparate Ags), although CD4+ lymphocytes participate in the pathogenesis of CAV, CD8+ lymphocytes can initiate CAV development independently of CD4+ lymphocyte help (44). Using a fully mismatched aortic graft model, Ensminger et al. have recently reported that CD8+ lymphocytes contribute to CAV development despite CD40L blockade (16). Only combination therapy with both anti-CD8+ lymphocyte-depleting Ab and CD40L-blocking Ab resulted in a significant reduction in CAV development. Jones et al. have also recently demonstrated that CD8+ lymphocytes can become activated, proliferate, and be recruited to cardiac allografts despite CD40L blockade (17). Taken together, these findings suggest that CD40L-CD40 interaction is an important signaling pathway when CD4+ lymphocyte help is absolutely required. In donor-recipient combinations, where disparate Ags are strong enough to stimulate CD8+ lymphocytes independent of CD4+ lymphocyte help, CD40L-CD40 signaling may not be required. CD8+ lymphocytes may use alternative costimulatory molecules, including ICOS, 4-1BB, and ICAM-1 (41–43). These findings have important clinical significance, suggesting that combination therapy (CD40L and CD8+ lymphocyte blockade) may be necessary for the prevention of chronic rejection.

In conclusion, this study on CAV provides new insights into the helper role provided by CD4+ lymphocytes, suggesting the importance of CD40L-CD40 signaling in an MHC class II-mismatched model. Current immunosuppressive regimens in clinical use fail to prevent chronic rejection, the major obstacle to long term patient survival. Improved understanding of the mechanism by which CD40L-CD40 signaling promotes chronic rejection may identify more specific targets for immunologic manipulation. Ultimately, this knowledge will assist in the development of novel treatment strategies for CAV and chronic rejection in other solid organ transplants.

References


43. Deets, M. J., and M. F. Mescher. 1999. ICAM-1 and B7-1 provide similar but distinct costimulation for CD8\(^+\) T cells, while CD4\(^+\) T cells are poorly costimulated by ICAM-1. Eur. J. Immunol. 29:45.