Impact of both donor and recipient strains on cardiac allograft survival after blockade of the CD40-CD154 costimulatory pathway
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famethoxazole-trimethoprim for several years, this medication was maintained unchanged throughout the course of her cutaneous eruption and its subsequent resolution.

The diagnosis of sirolimus-induced vasculitis was based on the temporal relationship between drug therapy and the biopsy-proven vascular inflammation, resolution of the inflammation upon discontinuation of drug therapy, and similar occurrences with reinitiating sirolimus. In light of our case report, sirolimus should be considered among the medications responsible for leukocytoclastic vasculitis.

Acknowledgments. The authors thank all of our transplant coordinators, especially Laura Roldan and Stacy Skelton, for their help in the care of our transplant patients.

REFERENCES

IMPACT OF BOTH DONOR AND RECIPIENT STRAINS ON CARDIAC ALLOGRAFT SURVIVAL AFTER BLOCKADE OF THE CD40-CD154 COSTIMULATORY PATHWAY

ANDRE VAN MAURIK,2 KATHRYN J. WOOD,2 AND NICK D. JONES2

Background. The effectiveness of anti-CD154 monoclonal antibodies in prolonging the survival of mouse allografts is dependent on the strain combination. In this report, we examined the impact of the donor and the recipient strains on the success of CD40-CD154 blockade.

Materials and Methods. Cardiac allograft survival was monitored in different donor/recipient strain combinations. Morphometric analyses on the allograft coronary arteries allowed quantification of vessel intimal thickening.

Results. Prolonged cardiac allograft survival after the administration of an anti-CD154 monoclonal antibody was found to be dependent on the donor and the recipient strains. The influence of the donor and the recipient strains lay in the ability of CD8+ T cells to cause graft rejection despite CD40-CD154 blockade. Elimination of CD8+ T cells before transplantation resulted in similar graft prolongation irrespective of the genotype of the donor or the recipient strain.

Conclusion. These data show that both donor and recipient strains contribute to CD40-CD154-independent CD8+ T-cell-mediated rejection.

Blockade of the CD40-CD154 pathway alone or in combination with blockade of the CD28/B7 costimulatory pathway has been shown to prolong the survival of allografts in rodents (1) and primates (2). Trambley et al. (3) recently reported that different mouse strain combinations showed greatly disparate skin allograft survival after blockade of the CD154 and CD28 pathways. Furthermore, the inability of the combined blockade to prevent skin allograft rejection in some strain combinations was caused by the activation of CD154/CD28-independent asialo GM1+ CD8+ T cells. We have previously also identified CD154-independent CD8+ T cells as being responsible for the failure of anti-CD154 monoclonal antibody (mAb) therapy to induce prolonged survival of cardiac allografts in certain strain combinations (4).

The objective of this investigation was to determine whether the donor or the recipient strain, or both, had an impact on the CD154-independent rejection of cardiac allografts and whether CD8+ T cells were responsible for graft rejection. Data that suggests that only the recipient strain influences graft outcome in this setting have been reported (5), but the data presented in this study clearly demonstrates that the donor genotype also has an impact on graft survival. In addition, we determined whether the donor or the recipient strain genotype had any impact on the well-documented

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DOI: 10.1097/01.TP.0000026578.98743.7E
development of graft arteriosclerosis in allografts that survive for more than 100 days after anti-CD154 mAb therapy.

MATERIALS AND METHODS

Mice

CBA.Ca (CBA; H2a), C57BL/10 (B10; H2b), BALB/c (H2d), and B10.BR (H2k) mice were purchased from Harlan Ltd. (Bicester, United Kingdom). B10.S(7R) (H2s) mice were a generous gift from B. Fazekas St. Groth, Sydney, Australia. All mice were bred and housed in the Biomedical Services Unit at John Radcliffe Hospital, Oxford, UK, in accordance with the Animal (Scientific Procedure) Act of 1986 of the UK. All experiments were performed with mice between 6 and 12 weeks of age.

Surgical Procedures and Antibody Treatment

Vascularized heterotopic heart grafts were performed as described previously (4). Graft survival data were analyzed by the log-rank method with a statistical application developed and kindly provided by S. Cobbold, University of Oxford, UK. P<0.05 was considered statistically significant.

Animals were treated with anti-CD154 mAb (MR1; American Type Culture Collection, Rockville, USA) or hamster control antibody (Jackson Immunoresearch, West Grove, PA) at 500 μg/day intraperitoneally (IP) on days 0, 2, and 4 after transplantation. For the depletion of CD8+ T cells, recipients were thymectomized and housed in the Biomedical Services Unit at John Radcliffe Hospital, Oxford, UK, in accordance with the Animal (Scientific Procedure) Act of 1986 of the UK. All experiments were performed with mice between 6 and 12 weeks of age.

Morphometric Analysis of Cardiac Grafts

Morphometric analysis to determine the degree of intimal thickening of the coronary arteries was performed as described previously (6).

RESULTS

To explore whether the prolongation of cardiac allograft survival by CD154 blockade was dependent on the donor strain, we transplanted BALB/c (H2b), B10 (H2b), or B10.S(7R) (H2s) fully MHC-mismatched cardiac allografts into CBA (H2a) mice. Recipients were treated with 500 μg/dose of an anti-CD154 mAb (MR1) on the day of transplantation and 2 and 4 days after transplantation. We found that CBA recipients treated with MR1 showed moderate prolongation of BALB/c (42%>100 days) and B10.S(7R) (14%>100 days) cardiac allograft survival. In contrast, 94% of B10 allografts in CBA recipients treated with MR1 accepted their grafts for more than 100 days (Table 1). BALB/c, B10, and B10.S(7R) cardiac allografts transplanted into CBA recipients treated with a control hamster antibody were rejected promptly with a median survival time (MST) of 7 to 8 days. The impact of the donor strain on the ability of CD154 blockade to prolong graft survival was confirmed in a second H2k recipient strain, namely B10.BR (Table 1). These results clearly demonstrate that, in CBA and B10.BR recipients, the genotype of the organ donor influences graft survival after anti-CD154 mAb treatment.

Although therapies that block costimulatory molecules inhibit allograft rejection in some mouse strains, it has been reported that this strategy is relatively ineffective in promoting allograft acceptance in other recipient strains (3, 5). This was also demonstrated in this study. As shown, MR1-treated CBA recipients accepted 94% of B10 and 42% of BALB/c cardiac allografts indefinitely (Table 1). Only 56% of B10.BR recipients treated with MR1 accepted B10 grafts, whereas all BALB/c cardiac allografts were rejected (MST=36 days; Table 1). Interestingly, prolongation of B10.S(7R) cardiac allograft survival was similar in CBA and B10.BR recipients after CD154 blockade (MST 22 and 25 days, respectively).

We have previously shown that CD8+ T cells can be activated independently of CD40-CD154 costimulation (4) and that CD8+ T cells can mediate allograft rejection and contribute to the development of vasculopathy despite the blockade of CD154 (6). To evaluate whether CD8+ T cells also contributed to rejection when the donor strain (B10 vs. BALB/c) influenced rejection, CBA recipients were thymectomized and depleted of CD8+ T cells. CD154 blockade was found to be equally effective in prolonging the survival of B10 and BALB/c cardiac allografts in this situation (B10 and BALB/c MST>100 days; Table 2).

Cardiac allografts that had survived 100 days in CBA recipients were then analyzed histologically with elastin van Gieson stain to assess vessel intimal thickening. As shown in Figure 1(A), the percentage of intimal thickening in B10 and BALB/c cardiac allografts was identical when CD8+ T cells were depleted in MR1-treated CBA recipients. Taken together, these data show that the disparity in allograft survival that was observed when cardiac allografts from different donor strains were transplanted into anti-CD154-treated recipients lay in the ability of CD8+ T cells to cause graft rejection; however, the development of arteriosclerosis re-

### Table 1. Effect of CD154-blockade on prolongation of cardiac allografts in different donor recipient strain combinations

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Treatment</th>
<th>Graft survival (days)</th>
<th>MST (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>CBA</td>
<td>MR1</td>
<td>12, 14, 37, 57, 69, 76, 96, &gt;100 (5)</td>
<td>86</td>
</tr>
<tr>
<td>BALB/c</td>
<td>CBA</td>
<td>H1g</td>
<td>7, 7, 8, 8</td>
<td>8</td>
</tr>
<tr>
<td>BALB/c</td>
<td>B10.BR</td>
<td>MR1</td>
<td>11, 24, 32, 36, 38, 44, 73</td>
<td>36</td>
</tr>
<tr>
<td>BALB/c</td>
<td>B10.BR</td>
<td>H1g</td>
<td>6, 6</td>
<td>6</td>
</tr>
<tr>
<td>B10</td>
<td>CBA</td>
<td>MR1</td>
<td>14, &gt;100 (15)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>B10</td>
<td>CBA</td>
<td>H1g</td>
<td>6, 6, 7, 7</td>
<td>7</td>
</tr>
<tr>
<td>B10</td>
<td>B10.BR</td>
<td>MR1</td>
<td>20, 56, 62, 83, &gt;100 (5)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>B10</td>
<td>B10.BR</td>
<td>H1g</td>
<td>7, 13</td>
<td>10</td>
</tr>
<tr>
<td>B10.S(7R)</td>
<td>CBA</td>
<td>MR1</td>
<td>14, 16, 19, 22, 39, 75, &gt;100</td>
<td>22</td>
</tr>
<tr>
<td>B10.S(7R)</td>
<td>CBA</td>
<td>H1g</td>
<td>8 (3)</td>
<td>8</td>
</tr>
<tr>
<td>B10.S(7R)</td>
<td>B10.BR</td>
<td>MR1</td>
<td>17, 19, 24, 25, 33, 50, 75</td>
<td>25</td>
</tr>
<tr>
<td>B10.S(7R)</td>
<td>B10.BR</td>
<td>H1g</td>
<td>6, 7, 7</td>
<td>7</td>
</tr>
</tbody>
</table>

* P<0.05.

* Not significant (P>0.05).
Table 2. Effect of CD154-blockade on prolongation of cardiac allografts in different donor recipient strain combinations in the absence of CD8+ T cells

<table>
<thead>
<tr>
<th>Donor</th>
<th>Recipient</th>
<th>Treatment</th>
<th>Graft survival (days)</th>
<th>MST (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BALB/c</td>
<td>CBA</td>
<td>CD8-depl. + MR1</td>
<td>13,22,28,57,72,79,87, &gt;100 (23)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>B10+</td>
<td>CBA</td>
<td>CD8-depl. + MR1</td>
<td>&gt;100 (5)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>BALB/c</td>
<td>CBA</td>
<td>CD8-depl. + HIg</td>
<td>10, 10, 11</td>
<td>10</td>
</tr>
<tr>
<td>BALB/c</td>
<td>B10.BR</td>
<td>CD8-depl. + MR1</td>
<td>12, 16, 33, 49, 55, 68, &gt;100 (5)</td>
<td>68</td>
</tr>
<tr>
<td>BALB/c</td>
<td>B10.BR</td>
<td>CD8-depl. + HIg</td>
<td>6, 8</td>
<td>7</td>
</tr>
<tr>
<td>B10.8/7R</td>
<td>B10.BR</td>
<td>CD8-depl. + MR1</td>
<td>9, 24, 35, &gt;100 (7)</td>
<td>&gt;100</td>
</tr>
<tr>
<td>B10.8/7R</td>
<td>B10.BR</td>
<td>CD8-depl. + HIg</td>
<td>7, 9</td>
<td>8</td>
</tr>
</tbody>
</table>

*Not significant (P>0.05).

a depl., depleted.

Figure 1. Morphometric analysis of the degree of intimal thickening of the coronary arteries of cardiac grafts 100 days after transplantation. Measurements of all coronary arteries from three to five different areas from the middle of the heart were obtained for this analysis. At least three separate cardiac grafts per group were evaluated. Mean percentage of intimal thickening for each group was statistically compared between groups with a standard t test. P<0.05 was considered significant. (A) Cardiac allografts from different donor strains transplanted into CBA recipients treated with MR1 that survived for 100 days were analyzed for the degree of intimal thickening. Cardiac allografts from either a BALB/c (open bar) or a B10 (black bar) donor transplanted into MR1-treated CBA recipients showed an identical degree of intimal thickening when host CD8+ T cells were depleted before transplantation (P=0.64). (B) BALB/c cardiac allografts transplanted into different CD8+ T-cell-depleted recipient strains (CBA and B10.BR) treated with MR1 did not show a significant difference in the degree of intimal thickening (open bars; P=0.11). Additional weekly administration of MR1 in these recipients did not reduce the degree of intimal thickening significantly (hatched bars; BALB/c to CBA P=0.59, BALB/c to B10.BR P=0.32). Control BALB/c hearts transplanted into BALB/c recipients did not show any sign of vasculopathy when analyzed 100 days after transplantation. n.s., not significant (P>0.05).

Discussion

Recently, it has been shown that the success of the blockade of the CD40 and CD28 pathway in a skin allograft model is solely dependent on the recipient mouse strain (5). In this study, we demonstrated that the donor strain can also play a significant role (Table 1). This finding has important implications for the development of reagents that block CD40-CD154 interactions as a therapeutic strategy to combat rejection in clinical transplantation.

We and others have identified CD154-independent CD8+ T cells as being responsible for the failure of anti-CD154 mAb therapy to induce prolonged allograft survival in certain strain combinations (3, 4, 7). Recently, the growth factor interleukin (IL)-15 has been reported to play a critical role in the costimulation of blockade-resistant CD8+ T-cell-mediated rejection (8); however, the exact activation requirements for these cells remain elusive.

The present study clearly demonstrates that the elimination of CD8+ T cells before transplantation results in a comparable prolongation of cardiac allografts irrespective of the donor or the recipient strain (Table 2). This finding indicates that donor and recipient differences, in terms of CD40-CD154-independent rejection, reside in the ability of CD8+ T cells to mediate graft rejection. These results are in agree-
ment with findings reported by Williams et al. (5). They also found that the ability of certain recipient mouse strains to reject MHC-mismatched skin allografts independently of CD28 or CD154 interactions could be attributed to the successful generation of cytotoxic T lymphocytes under these conditions. However, they failed to demonstrate any influence of the donor strain on CD28-CD154–independent rejection.

A possible explanation for our observation that the donor strain also has an influence on graft prolongation after costimulation blockade may lie in the differential susceptibility of different transplanted organs to rejection. It has been well established that skin allografts are more susceptible to rejection than cardiac allografts (9). It is therefore likely that donor-reactive CD8+ T cells, which have escaped costimulation blockade, will reject a susceptible graft, such as skin, acutely with similar kinetics irrespective of donor strain. A less susceptible graft, such as heart, may be more likely to reveal subtle changes in the kinetics of graft rejection between different donor strains.

Dissimilar survival of B10, BALB/c, and B10.S(7R) cardiac allografts is likely to be a result of immune gene polymorphisms. Polymorphisms in donor-derived factors, such as cytokines, chemokines, and their receptors and costimulatory molecules, may therefore confer quantitatively or qualitatively different responses.

In addition to similar graft prolongation after the depletion of CD8+ T cells, transplanted hearts showed identical development of vasculopathy irrespective of the donor or recipient strain. A potential mechanism for the development of vasculopathy in these recipients is immune deviation toward a T-helper cell 2 phenotype. We have previously shown, in an aortic allograft model, that in the absence of CD8+ T cells, inhibition of the CD40-CD154 pathway resulted in an augmented intragraft production of IL-4 and an increased presence of eosinophils (6, 10). Moreover, neutralizing IL-4 greatly reduced the eosinophil infiltrate and reduced graft vasculopathy.

In conclusion, the present study clearly demonstrates that the donor and the recipient strains contribute to CD40-CD154-independent CD8+ T-cell-mediated rejection. Our data also suggest that in the absence of CD8+ T cells, anti-CD154 mAb therapy is effective in the prevention of acute rejection. However, graft arteriosclerosis was not entirely prevented in the absence of CD8+ T cells.

REFERENCES