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T cell depletion eliminates the development of cardiac allograft vasculopathy in mice rendered tolerant by the induction of mixed chimerism.

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Abstract

We previously demonstrated that cardiac allografts to fully tolerant chimeric mice developed cardiac allograft vasculopathy (CAV). Here we begin to examine which components of the immune system are responsible for the pathogenesis of CAV in such tolerant recipients. B10.A/B6 mixed chimeric mice were created by receiving injections of bone marrow cells from B10.A (H-2^k) mice given to C57BL/6 (B6; H-2^b) mice with some preparations. B10.A skin grafts were first placed onto B10.A/B6 mixed chimeric recipients. When the donor strain skin grafts had survived perfectly for at least 56 days, B10.A hearts were transplanted heterotopically into B10.A/B6 mixed chimeric recipients. Hearts were examined for the presence of CAV 56 days later. To determine the effector cells that contribute to the development of CAV, they were treated weekly with a combination of anti-CD4/CD8 mAbs or anti-NK1.1mAb continuing until 56 days. 14 B10.A cardiac transplants of 18 otherwise untreated B10.A/B6 chimeric recipients developed CAV concurrent B6 isografts were unaffected (0/7). In chimeric recipients treated with anti-CD4/8 mAbs, the prevalence of CAV was greatly reduced (0/6, $p<0.01$ compared to the untreated group). Anti-NK1.1 mAb was not effective in the prevention of CAV (4/5). These data suggest that T cells may contribute in some way to the development of CAV that occurs in those fully tolerant recipients. Host T cells which may still be responsive to non-MHC antigens, including tissue specific antigens presented not on skin but on heart, may also be responsible for the development of CAV in tolerant animals.

Introduction

We previously demonstrated that cardiac allografts to mixed chimeric mice developed cardiac allograft vasculopathy (CAV), even though they were tolerant of donor antigens confirmed by donor strain skin graft survival (1). These findings suggested that innate immune responses might be involved in the process. Eventually, it has been shown that NK cells in mixed chimeras retained reactivity to host antigens and host NK cells were not tolerant of the donor (2). Although the elimination of host anti-donor T cell activity has been demonstrated in stable

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mixed chimeras (3), there is still a possibility that host T cells may be responsive to donor peptides presented through the indirect pathway or to non-MHC antigens (4, 5), including autologous tissue specific antigens, presented on the donor heart, such as cardiac myosin (6, 7). In this study, we have begun to examine which components of the immune system are responsible for the pathogenesis of CAV in such tolerant recipients in depletion studies.

Methods

B10.A/B6 mixed chimeric mice were created by receiving injections of anti-CD4/CD8 mAbs and CD40L mAb, 3Gy WBI, and bone marrow cells from B10.A (H-2^k) mice given to C57BL/6 (B6; H-2^b) mice as previously described (1, 8, 9). At 8 weeks after the induction of mixed chimerism, B10.A skin grafts were placed onto B6/B10.A chimeric recipients to confirm that they were tolerant of donor antigens in vivo. In addition to skin graft transplantation, flow cytometric analysis for the presence of multilineage chimerism in peripheral blood lymphocytes was performed. The percentages of donor derived B cells and granulocytes were 36–80% and 38–58%, respectively. Only mice that developed chimersim confirmed by FACS analysis and allowed the survival of these grafts in perfect condition for more than 56 days were admitted to the study. Finally, B10.A hearts were transplanted heterotopically into B6/B10.A chimeric recipients 16 weeks after the induction of chimerism. These were examined histologically for the presence of CAV 56 days later. To determine effector cells that contribute to the development of CAV in chimeric recipients, some recipients received injections of ascites fluid containing either 200 μl anti-CD4/CD8 mAbs or 200μl anti-NK1.1mAb beginning 7 days before heart transplantation (HTx) and continuing until 56 days on every week. Host T cell reactivity was measured at the end of the experiment by MLR assay using splenocytes from recipients (8). The significance of differences between groups was determined using Fisher's exact test.

Results

At 56 days after heart transplantation, anti-CD4/CD8 mAbs and anti-NK1.1 mAb treatment achieved depletion of more than 87% of splenic CD3 positive cell and 90% of splenic DX5 positive cell in B10.A/B6 chimeric recipients, respectively. In B6/B10.A chimeric mice, B10.A donor cell chimerism persisted during the experimental period. B10.A heart grafts continued to beat vigorously throughout the 56 day observation period. However, 14 B10.A cardiac grafts removed from 18 otherwise untreated B10.A/B6 mixed chimeric recipients developed florid CAV lesions while concurrent B6 isografts were not affected (0/7) (Table 1). When chimeric recipients were treated with anti-CD4/8 mAbs, the prevalence of CAV was completely prevented (0/6, $p<0.01$ as compared to the untreated group). Anti-NK1.1 mAb treatment was not effective in preventing CAV (3/4), suggesting that T cells were involved in the process of CAV formation in this setting. In MLR assay, splenocytes of B10.A/B6 chimeric mice did not show reactivity against B10.A donor antigens during the post B10.A heart transplant period. Therefore, it may be that these T cells contribute to the development of CAV not as a consequence of recognizing donor MHC antigens, but of recognizing antigens that are specific to cardiac tissue.

Conclusion

It has been reported that host anti-donor T cell reactivity is eliminated in stable mixed chimerism(10). However, these data suggest that T cells may contribute in some way to the development of CAV that occurs in mixed chimeric recipients. Host T cells may still be responsive at a low level to donor MHC-determined antigens presented through the indirect pathway or to non-MHC antigens, including tissue specific polymorphic antigens presented not on skin but on heart. It is also formally possible that donor T cells may induce a local GvH

response that leads to vasculopathy. Further studies will be necessary to clarify the mechanism of this process.

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Table 1

Hearts transplanted into mixed chimeric recipients treated with anti-CD4/CD8 mAbs or anti-NK1.1 mAb.

Transplant system	Treatment	No. of hearts with CAV	Statistical difference[a]
B10.A to B10.A/B6 chimera	None	14/18	
	Anti-CD4/CD8 mAbs	0/6	$p<0.01$[b]
	Anti-NK1.1 mAb	3/4	N.S.[c]
B6 to B6	None	0/7	

[a] Statistical differences were determined according to the Fisher exact test.

[b] The p value for group with no treatment vs anti-CD4/CD8 mAbs treatment

[c] The p value for group with no treatment vs anti-NK1.1 mAb treatment; $p<0.05$