# Natural killer cells play a critical role in mediating inflammation and graft failure during antibody-mediated rejection of kidney allografts

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**While the incidence of antibody-mediated kidney graft rejection has increased, the key cellular and molecular participants underlying this graft injury remain unclear. Rejection of kidney allografts in mice lacking the chemokine receptor CCR5 is dependent on production of donor-specific antibody. Here we determine if cells expressing cytotoxic function contributed to antibody-mediated kidney allograft rejection in these recipients. Wild-type C57BL/6, B6.CCR5$^{-/-}$, and B6.CD8$^{-/-}$/CCR5$^{-/-}$ mice were transplanted with complete MHC-mismatched A/J kidney grafts, and intragraft inflammatory components were followed to rejection. B6.CCR5$^{-/-}$ and B6.CD8$^{-/-}$/CCR5$^{-/-}$ recipients rejected kidney allografts by day 35, whereas 65% of allografts in wild-type recipients survived past day 80 post-transplant. Rejected allografts in wild-type C57BL/6, B6.CCR5$^{-/-}$, and B6.CD8$^{-/-}$/CCR5$^{-/-}$ recipients expressed high levels of VCAM-1 and MMP7 mRNA that was associated with high serum titers of donor-specific antibody. High levels of perforin and granzyme B mRNA expression peaked on day 6 post-transplant in allografts in all recipients, but were absent in isografts. Depletion of natural killer cells in B6.CD8$^{-/-}$/CCR5$^{-/-}$ recipients reduced this expression to background levels and promoted the long-term survival of 40% of the kidney allografts. Thus, natural killer cells have a role in increased inflammation during antibody-mediated kidney allograft injury and in rejection of the grafts.**

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Whereas the advent of calcineurin inhibitor–based immunosuppression has decreased T cell–mediated graft rejection, the incidence of antibody-mediated graft injury and rejection has increased.[1–4] Antibodies are reported to be the cause of up to 50% of acute rejection episodes in kidney transplants and more than 60% of late graft failure.[3–5] The increased incidence and difficulty in treating antibody-mediating rejection have underscored the need to develop new strategies to identify the cellular and molecular components underlying antibody-mediated kidney graft rejection. Antibody-mediated rejection of kidney allografts has histopathologic features distinct from those of T cell–mediated graft injury.[6–8] These include neutrophil and macrophage margination in peritubular capillaries and often within the glomeruli. In many cases donor-specific antibody (DSA) does not mediate rejection as a solitary event, but induces the activation of other components of innate and adaptive immunity to contribute to the graft tissue injury. The presence of T cell infiltration and tubulitis is often observed in for-cause biopsies with diagnosis of antibody-mediated rejection.[5,8–10] Such mixed T cell– and antibody-mediated histopathology may occur in 10% to 90% of kidney graft biopsies that fulfill the Banff criteria for antibody-mediated rejection.[5,9] In addition, the presence of transcripts associated with natural killer (NK) cell activation in kidney graft biopsies during antibody-mediated rejection is often observed, suggesting a role for NK cells in the pathology.[11–13] The contribution of T cells, NK cells, or both to kidney graft injury during antibody-mediated rejection, however, remains poorly defined.

We have developed a unique mouse model of antibody-mediated rejection of kidney allografts.[14,15] CCR5-deficient allograft recipients have dysregulated DSA responses where high titers are generated within 2 weeks of transplantation. In contrast to wild-type C57BL/6 recipients of complete major histocompatibility complex (MHC)–mismatched kidney allografts, all CCR5$^{-/-}$ recipients reject the allografts between days 17 and 30 post-transplant, and this rejection is dependent on the production of DSA. We have recently reported the activation of CD8 T cells in CCR5$^{-/-}$ recipients of kidney allografts,[16] but the contributions of CD8 T cells as well as NK cells during antibody-mediated rejection in these recipients have not been directly tested. In light of clinical

studies suggesting a potential impact of donor-reactive T cells, NK cells, or both in antibody-mediated rejection of renal allografts, we generated CD8$^{-/-}$CCR5$^{-/-}$ mice to test the role of donor-reactive CD8 T cells in antibody-mediated kidney allograft rejection. The goal of the current study was to compare the cellular and molecular events occurring in a temporal manner during the rejection of kidney allografts with versus without CCR5$^{-/-}$ recipient CD8 T cells, NK cells, or both. The results indicate that CD8 T cells are not required during antibody-mediated rejection of kidney allografts but that NK cells mediate intense intragraft inflammation and play a critical role in rejection.

## RESULTS

### Renal allograft survival in wild-type, CCR5$^{-/-}$, and CD8$^{-/-}$CCR5$^{-/-}$ recipients

A potential role for donor-reactive CD8 T cells during antibody-mediated rejection to renal allografts in CCR5-deficient recipients was investigated by comparing survival of complete MHC-mismatched A/J renal allografts in B6.CCR5$^{-/-}$ versus B6.CD8$^{-/-}$CCR5$^{-/-}$ recipients. Groups of wild-type C57BL/6 mice received A/J allografts or C57BL/6 isografts. C57BL/6 isografts were maintained long-term (>60 days) in wild-type C57BL/6 recipients without any incidence of graft failure (Figure 1a) as well as in B6.CCR5$^{-/-}$ and B6.CD8$^{-/-}$CCR5$^{-/-}$ recipients (data not shown). Consistent with our previous studies, renal allografts were maintained in wild-type C57BL/6 recipients until day 30 post-transplant, when 40% were rejected by day 40 post-transplant, but the remaining 60% of the allografts survived with good function beyond day 70 post-transplant. B6.CCR5$^{-/-}$ recipients began rejecting A/J allografts on day 15 post-transplant, and all allografts were rejected by day 35 with a mean time survival of 16.75 $\pm$ 5.70 days. B6.CD8$^{-/-}$CCR5$^{-/-}$ recipients rejected the renal allografts with slightly delayed kinetics with a mean time survival of 27.33 $\pm$ 13.67 days that was not significantly different from allograft survival in B6.CCR5$^{-/-}$ recipients. Extensive histopathologic evidence of antibody-mediated rejection was

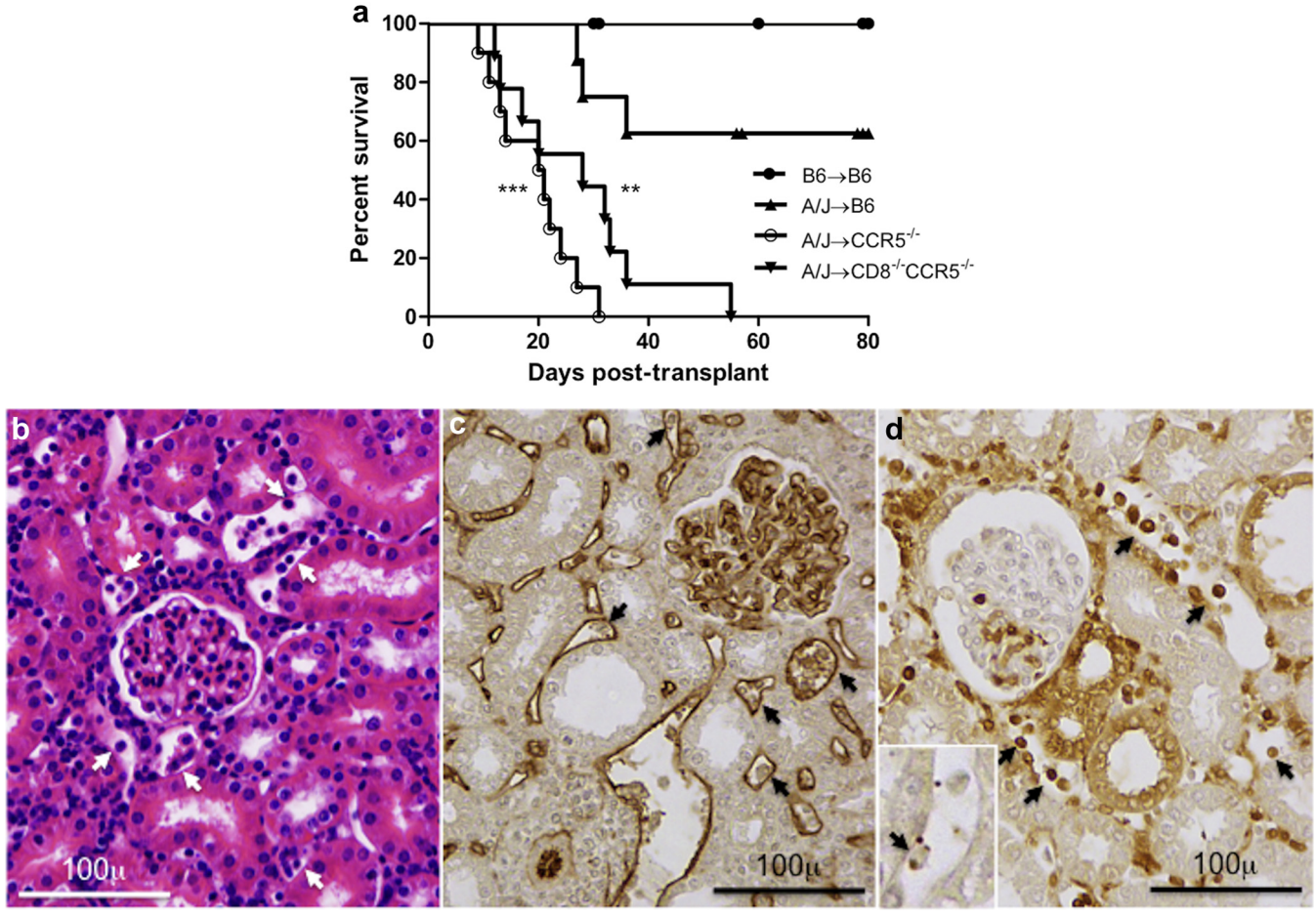


**Figure 1 | Survival of complete MHC-mismatched renal allografts in wild-type C57BL/6, B6.CCR5$^{-/-}$, and B6.CD8$^{-/-}$CCR5$^{-/-}$ recipients.** Groups of wild-type C57BL/6 ($n = 9$), B6.CCR5$^{-/-}$ ($n = 10$), and B6.CD8$^{-/-}$CCR5$^{-/-}$ ($n = 9$) mice received renal allografts from A/J donors. A group of wild-type C57BL/6 mice ($n = 5$) also received isografts. (**a**) Nephrectomy of the remaining native kidney was performed on day 5 post-transplant, and graft survival was followed by daily examination of overall animal health. $^{**}P < 0.01$, $^{***}P < 0.005$ compared with survival of allografts in wild-type C57BL/6 recipients. (**b–d**) Histologic evaluation of kidney allografts from B6.CCR5$^{-/-}$ recipients on day 6 post-transplant stained with (**b**) hematoxylin and eosin, (**c**) anti-C4d antibody, and (**d**) anti-Mac2 antibody. Arrows indicate marginating leukocytes. Images shown are representative of 3 individual allografts in the group.