

The Role of B Cell Mediated Antigen Presentation in Activation of Alloreactive CD4 T Cells and Allograft Rejection

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Activation of alloreactive CD4 T cells occurs via two distinct pathways of alloantigen presentation: the direct pathway, driven by graft derived antigen presenting cells (APCs), and the indirect pathway, mediated by recipient APCs. Macrophages and dendritic cells are thought to be dominant in priming self-restricted alloreactive CD4 T cells via the indirect pathway. However, despite the competence of B lymphocytes as potent APCs *in vivo*, their contribution to the indirect pathway of alloimmunity remained hitherto undefined. To determine the role of B cell APC function in alloimmunity, we generated mice with a selective deficiency in MHC class II mediated antigen presentation confined to B lymphocytes. Abrogation of B cell mediated indirect alloantigen presentation caused a marked prolongation of cardiac allograft survival, despite the competence of non-B cell APCs and T lymphocytes. We hypothesized that this finding is due to insufficient activation of alloreactive CD4 T cells resulting from absent B cell mediated antigen presentation. Here, we introduce a TCR/Ag transgenic murine model, which permits the clonotypic visualization of alloreactive CD4 T cell activation *in vivo*. We constructed BALB/c transgenic (Tg) mice that express the influenza virus hemagglutinin (HA) under the control of a MHC class II promoter. These HA Tg mice were used as donors of cardiac allografts. One day prior to transplantation, wild-type BALB/c recipients were inoculated with 2×10^6 CFSE-labeled transgenic CD4 T cells specific for a HA peptide presented in the context of BALB/c MHC class II I-Ed. We found that the HA-specific CD4 T cell response to the HA alloantigen derived from cardiac grafts began in the spleen, and was profoundly systemic by four days following transplantation. We sought to use this system to determine the role of indirect presentation in the activation of alloreactive CD4 T cells. To this end, HA Tg MHC class II^{-/-} mice were generated. In HA Tg MHC class II^{-/-} mice, HA is expressed on the surface of APCs that lack MHC class II. However, the inability of the grafts from HA Tg MHC class II^{-/-} mice to directly present HA did not diminish CD4 T cells activation by cardiac allografts. This finding demonstrated that the indirect pathway of alloantigen presentation is sufficient for alloreactive CD4 T cell activation in the case of cardiac allografts. Moreover, this transgenic system has allowed us to begin determining the contribution of B cell APC function to the activation of alloreactive CD4 T cells *in vivo*. Thus far, the present study has: 1) established the essential role of B cell mediated antigen presentation in allograft rejection and 2) developed a novel TCR/Ag transgenic system useful in delineating the contribution of B cells to CD4 T cell alloimmune responses *in vivo*.