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Summary
CAV is almost invariably associated with B cell infiltrates in or around coronary arteries as well as in endomyocardial tissue (quilty effect). It is assumed that these cells participate in the rejection process, although a formal demonstration of such contribution is still lacking. On the whole, infiltrating B cells are loosely defined. Their exact phenotype, antigen specificity and possible function are currently unknown. Understanding how B cells contribute to mechanisms of graft rejection would undoubtedly facilitate the development of therapeutic agents to target them and treat rejection. Studies in humans are challenging due to the limited source of samples and the difficulty of setting up techniques to study primary B cells retrieved directly from tissue. For these reasons, the assessment of human graft infiltrating B cells has been limited thus far and most studies have focused on humoral immunity using blood samples. We have begun collecting fresh cardiac graft specimens rejected because of CAV and explanted for re-transplantation through a collaborative network of transplant centers in the USA and Canada. Preliminary experiments using deep sequencing to analyze rearranged immunoglobulin heavy chain repertoire in situ demonstrated the massive expansion and somatic mutation of B cell clones in 4 cardiac allografts. We have also started immortalizing B cell clones directly from these cardiac allograft specimens. Our proposed research will characterize these cells, uncover their main function and evaluate their participation in the pathophysiology of CAV.
Aim 1. To characterize the phenotype, clonality and specificity of graft infiltrating B cells during CAV:
We will first assess the distribution and phenotype of B cells within the rejected graft tissue. B cell repertoire analyses will then identify predominant B cell clones expanded in situ. In parallel, B cells isolated from explanted grafts will be immortalized and cultured in vitro. Selected clones corresponding to B cells expanded in situ will be further characterized.
Aim 2. To identify the function of antibodies secreted by graft infiltrating B cells during CAV:
Monoclonal antibodies secreted by immortalized clones found to be expanded in situ in aim 1, will be assessed for their capacity to form immune complexes and induce cytokine secretion by macrophages in situ as well as facilitate antigen presentation to T cells.
Aim 3. To determine the function of graft infiltrating B cells during CAV:
Aside from their capacity to secrete pathogenic antibodies, we will examine whether graft-infiltrating B cells during CAV can uptake and present antigens to T cells. We will also investigate whether B cells can polarize T cell responses in situ. Lastly, we will also examine the role of the complement to modulate these responses.