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Review

B cells in cardiac transplants: From clinical questions to experimental models

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ABSTRACT

After many years of debate, there is now general agreement that B cells can participate in the immune response to cardiac transplants. Acute antibody-mediated rejection (AMR) is the best defined manifestation of B cell responses, but diagnostic and mechanistic questions still surround AMR. Many complement dependent mechanisms of antibody-mediated injury have been elucidated. C5 has become a therapeutic target that may not just truncate complement activation, but also may tip the balance away from inflammation by altering macrophage function. Additional complement independent effects have been identified. These may escape diagnosis and progress to chronic graft injury.

The function of B cell infiltrates in cardiac transplants is even more enigmatic. Nodular endocardial infiltrates that contain B cells and plasma cells have been described in protocol biopsies of cardiac transplants for decades, but an understanding of their significance is still evolving based on more critical morphological and molecular evaluation of these infiltrates. A range of infiltrates containing B cells has also been described in the epicardial fat in transplants with advanced chronic rejection. B cells have been observed in endocardial and epicardial tertiary lymphoid nodules, but their impact on antigen presentation or antibody production remains to be determined. Experimental models in small and large animals suggest that B cells could be essential for the formation of lymphoid nodules through cytokine production. Similarly, the role of proinflammatory adipokines in the formation or function of epicardial lymphoid nodules has not been studied.

These clinical observations provide critical questions to be addressed in experimental models.

1. Introduction

The potential effects of antibodies and B cell infiltrates on cardiac transplants have been the source of controversy for decades. Antibody-mediated rejection (AMR) was not accepted in the standardized grading system of the International Society for Heart and Lung Transplantation until 2004 [1]. Although many questions are not resolved, antibodies are now widely considered to cause injury and even rejection of some heart transplants [2,3]. Diagnosis of AMR is based on a triad of serological, histological and functional findings. The most generally recognized findings include donor specific antibody in the circulation, deposits of complement split products (C4d and/or C3d) in the capillaries of the biopsy and signs of cardiac dysfunction. Based on these criteria, AMR is diagnosed in about 1–10% of biopsies [2–4]. The debate now concerns whether AMR is more pervasive than is currently diagnosed. Arguments and mechanisms have been advanced for antibodies causing or at least contributing to rejection in the absence of one or more of the recognized criteria for AMR. For example, complement independent mechanisms of graft injury have been invoked in cases of graft dysfunction associated with circulating donor specific antibodies in the absence of C4d or C3d deposits [5]. Advances in knowledge about the effector mechanisms of antibodies are providing new insights to improve diagnosis and treatment of AMR. Therefore, one focus of this review will be effector mechanisms elicited by antibodies in transplants.

Similarly, nodular endocardial infiltrates containing B cells and plasma cells have been described in protocol biopsies of cardiac transplants since 1981 [6], but an understanding of their significance is still evolving based on more critical morphological and molecular evaluations of these infiltrates. A range of infiltrates containing B cells has also been described in the epicardial fat in transplants with advanced chronic rejection [7,8]. The potential importance of these endocardial and epicardial infiltrates will be a second focus of this review.

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The final focus of this review will be on experimental approaches to address evolving clinical questions about B cells in cardiac transplants.

2. New insights into antibody mediated rejection (AMR)

Cardiac transplants are closely monitored by protocol biopsies of the endocardium. The frequent biopsies provide an opportunity for assessing the occurrence of B cells and antibodies in symptomatic and asymptomatic cardiac transplants. However, diagnosis of AMR has been challenging because of the functional properties of antibodies. Although antibodies need to bind target antigens to initiate rejection, the antibodies only need to bind transiently in order to initiate a wide variety of inflammatory functions. The transient binding of antibodies makes them an elusive marker for AMR, and this was the basis for much of the controversy over early reports of AMR. However, the effects initiated by antibodies are more reliably assessed and more relevant to rejection. The most direct effects result from IgG or IgM antibodies cross-linking antigens on tissues. In addition, antibodies can activate the complement system and leukocytes. Whether one or more of these mechanisms is activated depends on many variables including the isotype, concentration, avidity and specificity of the antibodies. With increasing sophistication of serological tests, more data are available about these variables for circulating antibodies. However, it is not clear whether antibodies in the circulation accurately represent antibodies that are bound to the graft.

Increasingly detailed phenotypic and molecular studies of biopsies are providing greater insights about different effects of antibodies relative to activation of complement, leukocytes and endothelial cells. We will discuss each of these actions of antibodies in the following sections.

2.1. Complement activation

Activation of complement is the most extensively studied and diagnostically best characterized function of antibodies. In humans, IgG1 and 3 and to a lesser degree IgG2 can activate complement through C1 and Mannose Binding Lectin (MBL). The relative binding of C1 and MBL depends upon the structure of the carbohydrate side chains on the Fc of IgG antibodies [9]. The glycosylation of antibodies is not invariable. Variations in the carbohydrate structure of IgG autoantibodies have been recognized for many years. Autoantibodies that lack the terminal sialic acid and galactose (referred to as 'G0′ antibodies) expose terminal N-acetylglucosamine (GlcNAc) residues. Mannose binding lectin binds avidly to GlcNAc. As a result, G0 autoantibodies activate complement through the lectin pathway in both humans and mice [9,10]. The dynamics of glycosylation of antibodies requires further investigation, but it has been reported to be influenced by pregnancy and treatment with infliximab and possibly mycophenolate mofetil [11].

Detection of C1 or MBL has not been a sensitive or reliable indicator of AMR in biopsies. This is consistent with the facts that C1 and MBL are bound in lower concentrations than antibody (one C1 or MBL binds to 2 or more IgG molecules) and they are transiently bound to tissues through antibodies.

Both C1 and MBL enzymatically cleave numerous molecules of C4, and the C4 split product C4b can covalently bind to tissues. The biological importance of C4b is twofold. First, C4b is one of the ligands for complement receptor 1 (CR1; CD 35), which is expressed on circulating leukocytes. Second, C4b complexes with the split product of C2 to form the classical convertase that cleaves C3. C4b is important diagnostically because during regulation of the complement cascade, C4b is cleaved to an inactive split product C4d that remains covalently linked to the tissue. Moreover, the cleavage process reveals a cryptic epitope on C4d. As a result, monoclonal and polyclonal reagents to this cryptic epitope produce sensitive and specific immunohistological stains for complement activation (Fig. 1). However, the weak inflammatory properties of C4b and biological inactivity of C4d mean that positive stains for C4d do not necessarily correlate with rejection. In fact, C4 can moderate immune responses as evidenced by the fact that deficiencies in C4 are associated with increased autoimmune diseases. In transplantation, C4d deposits in the absence of accompanying inflammatory infiltrates are not associated with graft dysfunction, but with a state that has been termed accommodation. Accommodation has been most frequently observed in ABO blood group incompatible renal transplants. These transplants are feasible with living donors because they require extensive scheduled treatments of the recipient to decrease antibody titers before transplantation. ABO incompatible heart transplants are not intentionally performed with the notable exception of neonatal recipients. West and colleagues have successfully transplanted ABO incompatible hearts to infants before the age when they develop "natural" antibodies to A and B presumably in response to bacterial colonization of their gut. These transplants induce tolerance to the donor blood group [12]. The donor antigens remain expressed on the graft endothelium, but recipient B cells do not produce antibodies to donor or recipient blood group antigens.

Whether accommodation occurs in cardiac transplants remains to be determined, but Tan and colleagues have reported a cohort of adult recipients of ABO compatible cardiac transplants, whose endocardial biopsies have strong linear deposits of C4d in the absence of apparent graft dysfunction. Only a third of these patients had detectable circulating donor specific antibodies, and therefore, may represent a clinical situation in which low levels of antibodies activate complement at a gradual pace or in limited amounts that allow C4 to be continually inactivated before significant amounts of C3 are cleaved. In fact, many of these biopsies had detectable decay accelerating factor (DAF; CD55) and protectin (CD59), two complement regulatory molecules that have been shown to be upregulated in accommodation. DAF accelerates the disassociation of C3 convertase and leaves C4b attached to tissue. It is not known what mechanisms control the expression of complement regulators in transplants, but TNFα and IFNγ increase DAF expression by endothelial cells in vitro [13]. Mediators associated with chronic inflammation, such as basic FGF and VEGF also upregulate the expression of DAF.

Although structurally similar to C4, the split products of C3 have more inflammatory functions. Similar to C4b, C3b can bind covalently to tissues, but C3b represents a focal point of the complement system because C3b can initiate the alternative pathway of complement, which serves as an amplification loop. This can result in tenfold more C3b than C4b bound to the tissues, and greatly increases the density of ligands for CR1, which binds both C3b and C4b. Moreover, C3a, the small split product of C3, is a chemoattractant for neutrophils and macrophages and serves to bring these cells towards the tissue bound ligands. This is one mechanism to account for the association of neutrophils and macrophages with AMR.

The immunological balance is further shifted towards inflammation by the participation of C3b in the formation of C5 convertases with both classical and alternative complement components. These C5 convertases cleave C5 into C5a and C5b. C5b initiates the formation of the membrane attack complex (MAC; C5b-C9). This is an inefficient process and is countered by various regulators on allografts, including CD59. As a result, endothelial lysis is not widespread in AMR. However, sublytic amounts of MAC can activate endothelial cells as has been documented extensively using cell cultures and purified C5b through C9 [14–16]. The immediate effect of endothelial cell activation is retraction of the plasma