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Detection of alloantibody-mediated complement activation: A diagnostic advance in monitoring kidney transplant rejection?

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ABSTRACT

Objective: Antibody-mediated rejection (ABMR) is an important cause of kidney allograft injury. In the last two decades, detection of complement split product C4d along transplant capillaries, a footprint of antibody-mediated classical complement activation, has evolved as a useful diagnostic marker of ABMR. While it was recognized that ABMR may occur also in the absence of C4d, numerous studies have shown that C4d deposition may indicate a more severe rejection phenotype associated with poor graft survival. Such studies suggest a possible diagnostic benefit of *ex vivo* monitoring the complement-activating capability of circulating alloantibodies.

Design and methods: We reviewed the literature between 1993 and 2015, focusing on *in vivo* (biopsy work-up) and *in vitro* detection (modified bead array technology) of HLA antibody-triggered classical complement activation in kidney transplantation.

Results: Precise HLA antibody detection methods, in particular Luminex-based single antigen bead (SAB) assays, have provided a valuable basis for the design of techniques for *in vitro* detection of HLA antibody-triggered complement activation reflected by C1q, C4 or C3 split product deposition to the bead surface. Establishing such assays it was recognized that deposition of complement products to SAB, which critically depends on antibody binding strength, may be a cardinal trigger of the prozone effect, a troublesome *in vitro* artifact caused by a steric interference with IgG detection reagents. False-low IgG results, especially on SAB with extensive antibody binding, have to be considered when interpreting studies analyzing the diagnostic value of complement in relation to standard IgG detection. Levels of complement-fixing donor-specific antibodies (DSA) were shown to correlate with the results of standard crossmatch tests, suggesting potential application for crossmatch prediction. Moreover, while the utility of pre-transplant complement detection, at least in crossmatch-negative transplant recipients, is controversially discussed, a series of studies have shown that the appearance of post-transplant complement-fixing DSA may be associated with C4d deposition in transplant capillaries and a particular risk of graft failure.

Conclusions: The independent value of modified single antigen bead assays, as compared to a careful analysis of standard IgG detection, which may be affected considerably by complement dependent artifacts, needs to be clarified. Whether they have the potential to improve the predictive accuracy of our current diagnostic repertoire warrants further study.

1. Introduction

Antibodies directed towards incompatible polymorphic tissue antigens, most prominently human leukocyte antigens (HLA), may trigger tissue inflammation and injury leading to a progressive decline in allograft function and graft failure [1–3]. There are several lines of clinical evidence that antibody-mediated activation of the classical complement cascade, a process that may lead to the generation of a variety of biologically active molecules contributing to inflammation and tissue damage, may be an important effector mechanism [4,5]. It is well known that

Abbreviations: ABMR, antibody-mediated rejection; CDC, complement-dependent cytotoxicity; CDCXM, complement-dependent cytotoxicity crossmatch; DSA, donor-specific antibodies; DTT, dithiothreitol; EDTA, ethylenediaminetetraacetic acid; FCXM, flow cytometry crossmatch; HLA, human leukocyte antigen; LCL, lymphoblastoid cell lines; MFI, mean fluorescence intensity; PTC, peritubular capillaries; SAB, single antigen bead; XM, crossmatch.

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preformed donor-specific antibodies (DSA) with complement-activating capability, uncovered by complement-dependent cytotoxicity crossmatch (CDCXM), pose a high risk of hyperacute rejection [6]. Moreover, immunohistological detection of complement activation in allografts, reflected by the deposition of the classical C4 split product C4d along the transplant endothelium, was shown to associate with typical morphological and molecular features of antibody-mediated rejection (ABMR), graft dysfunction and increased rates of graft failure [7–10]. Finally, a recent uncontrolled study has suggested that targeted blockade of complement using a monoclonal anti-C5 monoclonal antibody may effectively prevent early antibody-mediated rejection (ABMR) in crossmatch (XM)-positive patients, even in the presence of high levels of DSA [11].

Presuming an important role of complement as an effector of rejection, the selective detection of antibodies that have the capacity to activate complement may be a good candidate for immune monitoring to assess the severity and prognosis of ABMR. *Ex vivo* detection of donor-directed complement activation requires the use of tools that allow for the detection of complement activation specifically targeted to alloantigens that are expressed also on the donor endothelium. In recent years, sensitive and specific solid phase assays based on the use of microbeads coated with defined HLA antigens have been developed [12–14]. The use of Luminex technology thereby allows for the detection of reactivity against a broad array of different HLA alleles within a single probe. Primarily, single antigen bead (SAB) arrays have been developed for the detection of reactivity against HLA antigens to define and characterize IgG type DSA. In addition, it has turned out that this diagnostic technology is applicable also for dissecting the ability of detected single reactivity to activate the classical complement pathway [15,16].

This review focuses on the concept of *in vivo* (biopsy work-up) and *in vitro* (modified SAB assays) monitoring the complement-activating capability of HLA antibodies discussing potential strengths and limitations of such assays in the clinical management of kidney transplant recipients.

2. Methods

Our review is based on a detailed search of the past and recent literature (1993–2015) conducted on the National Library of Medicine database, Pubmed. We used combinations of keywords including: antibody-mediated rejection, C4d, C1q, C3d, HLA antibody, single antigen bead, and kidney transplantation. For this review article, we also included relevant references cited in the selected papers.

2.1. Complement split product deposition in allografts

2.1.1. Capillary C4d — a footprint of alloantibody-mediated classical complement activation

As illustrated in Fig. 1, C4d is a degradation product resulting from cleavage of the classical complement component C4 that can be easily detected by immunohistology. Complement component C4 is activated by the C1 complex, which is attached to antigen-bound IgG. The proteolytically active form of C1s cleaves C4 into C4a and C4b. The larger split product C4b exposes an active thioester which is either immediately hydrolyzed by water molecules or binds covalently to hydroxyl or amino groups derived from carbohydrate moieties or peripheral membrane proteins of cell surfaces in its tight proximity. Together with C2a, surface-bound C4b forms the classical pathway C3 convertase, which is able to carry on the activation signal to C3 and other down-stream components. However, C4b is short-lived and is rapidly cleaved into C4d and nascent C4c (Fig. 1). For C4d, which remains covalently bound to the cell surface, no specific function has so far been documented. In transplantation medicine it is commonly regarded as a waste product indicating previous activation of the classical pathway [9].

2.1.2. Capillary C4d deposition — a marker of ABMR in kidney transplantation

In the early 1990s, Feucht et al. [7,17] were first to demonstrate that, applying immunofluorescent staining of transplant biopsies, a distinct proportion of renal allografts showed a capillary pattern of complement split product C4d deposition. They found that C4d

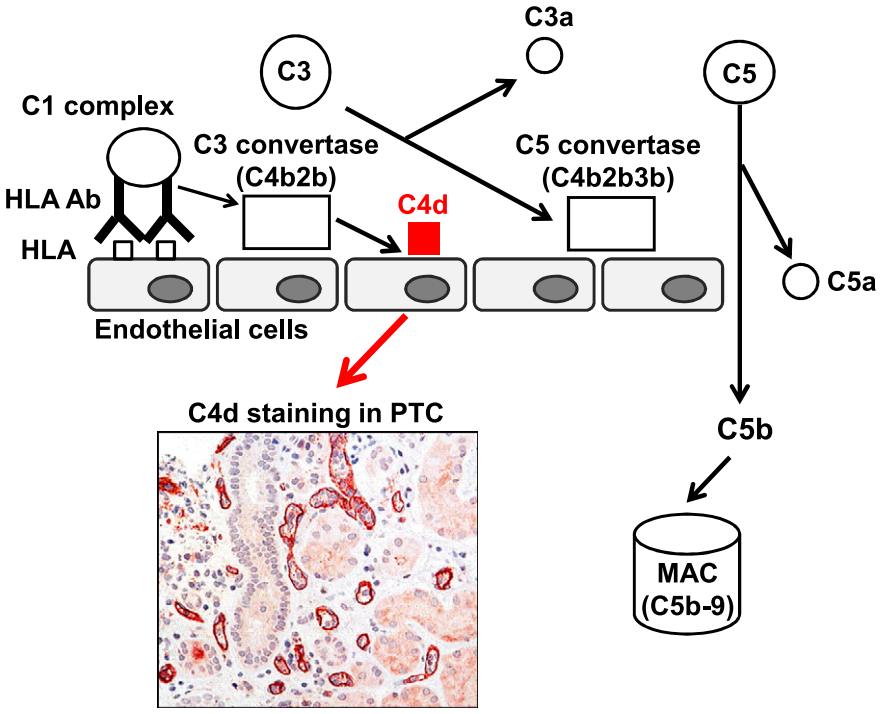

Fig. 1. Schematic illustration of alloantibody-triggered complement activation and C4d deposition. HLA antibody (Ab) binding to HLA antigens expressed on endothelial cells leads to activation of the classical complement cascade including covalent binding of C4b to the endothelial surface and further cleavage down to the still covalently bound end product C4d. In rejecting kidney allografts, C4d can be detected along capillaries. The figure shows a representative example of diffuse immunohistochemical C4d staining in peritubular capillaries (PTC).