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Protein kinase Cζ: not-so-innocent bystander or unusual suspect in kidney transplant rejection?

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Antibodies against non-HLA targets are increasingly recognized in the context of transplant rejection. However, their specific role remains largely elusive, as evidence exists supporting both their occurrence as an epiphenomenon and their actual pathogenicity in the rejection process. Sutherland *et al.* describe protein kinase Cζ as a novel, non-HLA antigenic target in the setting of graft rejection.

Kidney International (2009) **76,** 1223–1224. doi:10.1038/ki.2009.393

Some time ago the transplantation community moved past the notion that rejection is mediated exclusively via direct invasion of activated immune cells, that is, cytotoxic T lymphocytes in the allograft. Instead, acute cellular rejection is now known to often be accompanied by antibody-mediated phenomena, although occasionally antibody-mediated rejection can occur in the absence of acute cellular rejection. The antibodies involved in these phenomena were initially identified as targeting donor human leukocyte antigen (HLA) and causing damage by activating complement. This discovery resulted in techniques to better diagnose and characterize these antibody-mediated processes—for example, identification of donor-specific antibodies and titer quantification in recipient sera. As well as staining of biopsy tissue for C4d.

Recently, it has become increasingly clear that antibodies not directed against donor HLA and not causing complement-mediated lysis of target tissues may also play roles in the pathogenesis of acute and chronic graft damage.[1] Sutherland *et al.*[2] (this issue) present work that elaborates on this concept. Using protein microarray technology, they screened 15 pediatric kidney transplant recipients with acute cellular rejection (several with concomitant evidence of antibody-mediated phenomena, that is, positive C4d staining, detectable donor-specific antibodies, or antibodies directed against HLA that was not donor-specific) for *de novo* antibodies against 5056 potentially antigenic protein targets. They found *de novo* antibody formation against 229 non-HLA proteins, with antibodies against 36 of these targets detectable in more than one of the patients with rejection. Of all observed antibody responses, the strongest occurred against protein kinase Cζ (PKCζ) in three individuals. While C4d staining was negative in the biopsies of these patients, they had particularly recalcitrant clinical courses, and two of them lost their transplants within 2 years. In contrast, none of the 12 other patients with rejection but without a strong anti-PKCζ response experienced graft loss during follow-up.

The authors interpret their findings with appropriate caution given their relatively small patient-sample size and the retrospective structure of their study. Although they consider detectable anti-PKCζ to be a likely marker for rejection associated with poor graft survival, they do not believe that it is a pathogenic antibody. This notion of anti-PKCζ as a bystander is at least in part based on the authors' inability to detect C4d in the transplant biopsies of the rejection patients with a strong anti-PKCζ response. Accordingly, they hypothesize that, in these patients, severe renal injury and cell death led to PKCζ exposure and subsequent antibody formation (Figure 1, top).

Could anti-PKCζ be a novel mediator of an unusually aggressive subtype of rejection? This would be possible, if anti-PKCζ antibodies were activating similarly to those against angiotensin II receptors described by Dragun *et al.*[3] PKCζ plays critical roles in immune signaling pathways,[4,5] and its excessive

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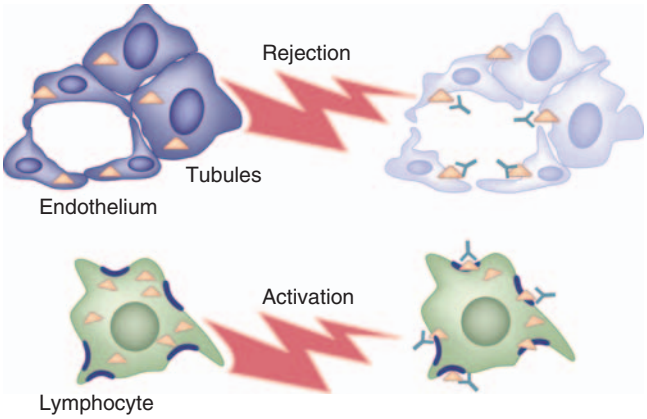

Figure 1 | Possible roles for PKCζ (yellow triangles) and antibodies against it (blue Ys) in kidney transplant rejection. Top: Donor PKCζ in the graft could become exposed as an antigenic target if inflammatory events during a rejection episode significantly impair tissue integrity. Bottom: Recipient PKCζ, after translocating into lipid rafts (curved blue disks) during immune activation, becomes accessible to potentially activating anti-PKCζ antibodies, providing a possible mechanism for amplification of the rejection response.

activation in recipient lymphocytes could thus contribute significantly to rejection. Along these lines, PKCζ has been shown to target to lipid rafts in macrophages stimulated via Toll-like receptor 2,[6] suggesting that the kinase could be more exposed as a target for antibodies in activated immune cells (Figure 1, bottom). However, evidence is lacking to support this concept in lymphocytes involved in allograft rejection.

Further, both processes could occur sequentially: Exposure of donor PKCζ in kidney tissue damaged by rejection could first lead to the generation of antibodies against the kinase. Subsequently, those antibodies could bind to and activate recipient PKCζ after its translocation to lipid rafts on the surface of immune cells participating in the rejection process. This activation could thus amplify the intensity of the rejection, explaining the particularly poor outcomes seen by Sutherland *et al.*[2] in patients with anti-PKCζ.

Clearly, future studies are needed to better understand the exact role of PKCζ and the antibodies against it (and other targets identified by the authors) in graft rejection. Such studies should clarify whether these antibodies are auto- or alloantibodies, that is, whether they are directed against the donor or recipient PKCζ (or both); whether they indeed activate the pathways; and whether they can modulate immune responses in manners not involving complement activation, as suggested by Sumitran-Holgersson[1] for other non-HLA antibodies. Lastly, it would be interesting to elucidate why some individuals generate significant titers of anti-PKCζ antibodies during acute rejection episodes whereas others do not.

The fascinating observations made by Sutherland *et al.*[2] with their innovative protein microarray approach provide new avenues of investigation that could lead to a better understanding of the role of non-HLA antibodies and their targets in graft rejection. Currently, we simply do not know yet whether these antibodies are conspicuous bystanders or pathogenic suspects in the immune responses involved in graft rejection.

DISCLOSURE

The author declared no competing interests.

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