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Serial monitoring of humoral antibody-mediated rejection of cardiac allografts by C4d staining of interstitial capillaries

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ABSTRACT

C4d "staining" of interstitial capillaries of endomyocardial biopsies serves as an indicator of a humoral component of cardiac allograft rejection. In the present study, two cardiac allograft recipients were monitored serially for both cellular and humoral rejection. Cellular rejection, evaluated by light microscopy, and humoral rejection, judged by C4d immunofluorescent "staining", were treated with appropriate immunosuppressants.

Weekly serial biopsies of the first patient revealed maximal humoral rejection (3+) after 1 week but diminished to 2+ thereafter. Cellular rejection was graded as 3A after 3 weeks but declined steadily to negligible cellular rejection through week 15. Whereas, cellular rejection peaked at 14–21 days, humoral rejection was greatest in the early post-transplant period. Biopsies of the second patient for 12 weeks revealed grade 1A to 1B moderate cellular rejection. Humoral rejection peaked at 3+ "staining" for C4d after 1 week transplant, and then wavered between 2+ (moderate "staining") and 1+ (weak "staining"). Results revealed the significance not only of traditional light microscopy in evaluating the severity of cellular rejection in endomyocardial biopsies of cardiac allotransplants, but also the value of C4d immunofluorescent "staining" of interstitial capillaries as an indicator of humoral rejection episodes, which may require modified therapy.

Introduction

C4d is a complement split product produced when C4bi is cleaved during a humoral immune response (Cruse and Lewis, 2004). C4d staining of peritubular capillaries by immunofluorescence technology has become an accepted method to monitor the development of a humoral antibody component of acute renal allograft rejection (Mauiyeddi and Colvin, 2002), which is principally cell-mediated (Fig. 1). Identification of an antibody mediated response against transplanted tissue is necessary to prevent changes in hemodynamics of the organ which may lead to increased mortality (Uber et al., 2007). However, there are instances in which a humoral antibody component of acute rejection is present in the absence of C4d staining of peritubular capillaries following renal transplantation (Colvin, 2007). The present study focuses on two cases where recognition of a humoral antibody component was detected by C4d staining. Use of this technique has been expanded to the examination of endomyocardial biopsies of cardiac allografts for evaluation of C4d staining of interstitial capillaries (Moll and Pascual, 2005). We have found C4d staining

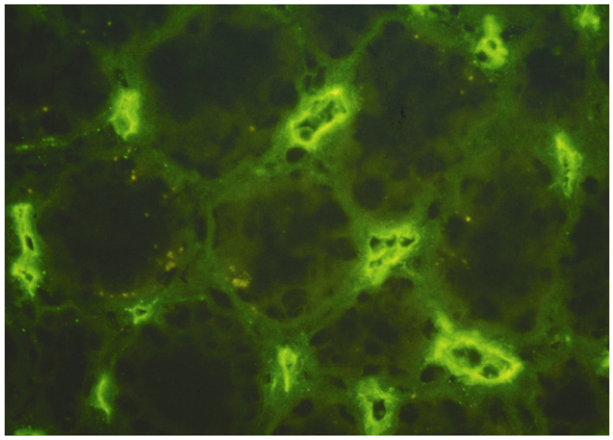

Fig. 1. Immunofluorescence 'staining' of C4d in peritubular capillaries in acute renal allograft rejection.

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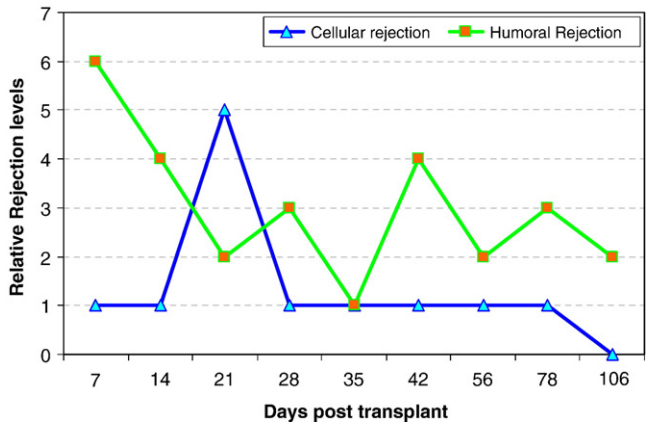

Fig. 2. Cellular and humoral rejection levels for patient 1 following cardiac allograft transplantation. Relative levels determined for cellular rejection as follows: 1A=1, 1B=2, 2A=3, 2B=4, 3A=5, and 3B=6. For humoral rejection, immunofluorescence staining of C4d rated negative=0; weak=1; "1+"=2; "2+"=4; "1+/2+"=3; "3+"=6.

to be just as revealing in monitoring the fate of cardiac allotransplants as it is in following renal allografts (Cruse et al., 2008). C4d staining can also be utilized to determine effectiveness of pharmacologic therapies used post-transplant in alleviating acute rejection (Crespo-Leiro et al., 2005). The present investigation was designed to determine whether or not serial monitoring of C4d staining of interstitial capillaries in endomyocardial biopsies could be useful in designing an appropriate immunosuppressive drug therapy for cardiac allotransplant recipients.

Methods

Standard immunohistochemical methods employed monoclonal antibodies against C4d and FITC-labeled rabbit anti-mouse IgG in a "sandwich" technique for C4d staining. Evaluation of morphologic criteria by light microscopy included quantification of the size of foci and determination of the number of foci with myocyte injury (graded as 2 and above, including 3a, 3b, and 4). Myocyte injury and size of lymphocyte foci were converted to numerical values, as described by the International Society for Heart and Lung Transplantation Working Formulation of Cardiac Allograft Pathology (ISHLT-WF1990). Immunofluorescence quantification was based on the ratio of vascular intensity to the background.

Results and discussion

The serial monitoring of C4d staining of interstitial capillaries in endomyocardial biopsies from two cardiac allograft recipients was used to help direct alterations in immunosuppressive therapy. The ability to ameliorate humoral antibody rejection episodes, in addition to the usefulness of standard light microscopy in evaluating the cellular limb of rejection, has facilitated cardiac allograft survival in the patients reported here. Therapeutic agents employed in this study included Cellcept®, Prograf®, prednisone, Cytoxan®, Solu-Medrol®, and Rapamune®.

In the first patient, 9 endomyocardial biopsies were evaluated for both cellular and humoral rejection (Fig. 2). Additionally, the therapeutic agents utilized were correlated with relative degree of rejection (Table 1). Immediately after transplantation, the patient began treatment with prednisone, mycophenolate mofetil (Cellcept®), and tacrolimus (Prograf®). After 7 days patient 1 exhibited a high degree of humoral rejection, as seen by a 3+ staining intensity of C4d by immunofluorescence (Fig. 3, part A) and a slight cellular rejection graded at 1A by H and E staining (Fig. 4, part A). Cyclophosphamide (Cytoxan®) was started, which appeared to alleviate the humoral rejection somewhat as seen by a 2+ staining intensity (Fig. 3, part B) on a biopsy from day 14 post-transplant, which continued to diminish to a 1+ intensity (Fig. 3, part C) on day 21 biopsy. This biopsy revealed, however, a grade 3A cellular rejection (Fig. 4, part C), which prompted an increased dosage of tacrolimus and a short course of methylprednisolone (Solu-Medrol®). Biopsy from day 28 to 106 post-transplant demonstrated a diminished cellular rejection (grade 1A or less), while humoral rejection levels varied. C4d staining intensity on day 28 ranged from 1+ to 2+ (Fig. 3, part D) which prompted an increase in cyclophosphamide dosage. The addition of sirolimus (Rapamune®) did not appear to control the humoral rejection, as seen by staining intensities of 2+ on day 42 (Fig. 3, part F), 1+ on day 56 (Fig. 3, part G), 1+ on day 78 (Fig. 3, part H), and 1+ on day 106 post-transplant.

The combination of tacrolimus and methylprednisolone proved to be effective in decreasing the levels of cellular rejection in the patient, while cyclophosphamide attenuated humoral aspects of rejection.

In the second patient, 8 endomyocardial biopsies were evaluated for cellular and humoral rejection (Fig. 5), and similar drug therapies were employed (Table 2). Immediate treatment with mycophenolate mofetil, tacrolimus and prednisone did not prevent an early humoral rejection, as seen by immunofluorescent C4d staining of biopsies taken 7 days post-transplant (Fig. 6, parts A–C). Addition of cyclophosphamide and an increase in tacrolimus diminished humoral rejection by day 14 (1+ staining intensity, Fig. 6, parts D–E). Humoral and cellular rejection both increased on day 21 post-transplant to 2+ staining intensity for C4d (Fig. 6, parts F–G) and 1B grade rejection on H and E staining (Fig. 4, part B), respectively. At this point tacrolimus was increased again, and a short course of higher dose prednisone was incorporated into the drug regime. Biopsies from day 28 (Fig. 6, part H) throughout revealed negligible humoral rejection, and biopsies from day 42 throughout demonstrated control of cellular rejection. The combination of tacrolimus with cyclophosphamide attenuates humoral rejection, while tacrolimus with high dose

Table 1
Post-transplant therapeutics used by patient 1

Days (post-transplant)	Cellcept (Mycophenolate mycophenolate mofetil)	Cytoxan (cyclo-phosphamide)	Prograf (tacrolimus)	Prednisone	Rapamune (sirolimus)	Solu-medrol (methyl-prednisolone)
0–7	Yes		2 mg, BID	20 mg, daily		
8–21		50 mg, daily	2 mg, BID	20 mg, daily		
22–24		50 mg, daily	3 mg, BID	20 mg, daily		1 g daily
25–28		50 mg, daily	3 mg, BID	20 mg, daily		
29–35		75 mg, daily	2 mg, BID	17.5 mg daily		
36–42		75 mg, daily	2 mg, BID	15 mg daily	1 mg daily	
43–56		50 mg, daily	2 mg, BID	12.5 mg daily	2 mg daily	
57–78		50 mg, daily	2 mg, BID	10 mg daily	2 mg daily	
79–94		50 mg, daily	3 mg AM/2 mg PM	7.5 mg daily	2 mg daily	
95–106		50 mg, daily	2 mg, BID	7.5 mg daily	2 mg daily	