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Prospective assessment of antidonor cellular alloreactivity is a tool for guidance of immunosuppression in kidney transplantation

Oriol Bestard[1,2], Josep M. Cruzado[1,2], Marc Lucia[2], Elena Crespo[2], Linda Casis[2], Birgit Sawitzki[3], Katrin Vogt[3], Carme Cantarell[4], Joan Torras[1,2], Edoardo Melilli[1], Richard Mast[5], Alberto Martinez-Castelao[1], Montse Gomà[6], Petra Reinke[3], Hans-Dieter Volk[3] and Josep M. Grinyó[1,2]

[1]Renal Transplant Unit, Nephrology Department, Bellvitge University Hospital, Barcelona, Spain; [2]Experimental Nephrology Department, IDIBELL, Barcelona University, Barcelona, Spain; [3]Berlin-Brandenburg Center for Regenerative Therapies (BRCT), Charité-Universitätmedizin Berlin, Campus Virchow-Klinikum, Berlin, Germany; [4]Renal Transplant Unit, Vall Hebrón University Hospital, Barcelona, Spain; [5]Radiology Department, Bellvitge University Hospital, Barcelona, Spain and [6]Pathology Department, Bellvitge University Hospital, Barcelona, Spain

Current characterization of the immune risk in renal transplant patients is only focused on the assessment of preformed circulating alloantibodies; however, alloreactive memory T cells are key players in mediating allograft rejection. Immune monitoring of antidonor alloreactive memory/effector T cells using an IFN-γ Elispot has been shown to distinguish patients at risk for immune-mediated graft dysfunction, suggesting a potential tool for immunosuppression individualization. In this nonrandomized study, we prospectively assessed donor and nondonor T-cell alloreactivity in 60 highly alloreactive patients receiving calcineurin inhibitor-based immunosuppression and in non-T-cell alloreactive transplant recipients treated with a calcineurin inhibitor-free regimen. The impact was evaluated using 1-year allograft outcome. We found a strong association between ongoing antidonor T-cell alloreactivity and histological lesions of acute T cell–mediated rejection in 6-month protocol biopsies, distinguishing those patients with better 1-year graft function, regardless of immunosuppression regimen. Interestingly, evidence for enhanced immune regulation, driven by circulating Foxp3-demethylated regulatory T cells, was only observed among patients achieving antidonor T-cell hyporesponsiveness. Thus, prospective evaluation of donor-specific T-cell sensitization may add crucial information on the alloimmune state of transplanted patients to be used in daily clinical practice.

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Correspondence: *Oriol Bestard, Renal Transplant Unit, Nephrology Department, Bellvitge University Hospital, 08907 Barcelona, Spain. E-mail: 35830obm@comb.cat*

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Although outstanding progress has been made in the last decades in the understanding of the intricate molecular mechanisms of allograft rejection and immunosuppression, long-term results and concerns regarding life-long immunosuppression still remain.[1–3] Differently from the majority of other diseases, in organ transplantation (TX) decision-making regarding type and amount of immunosuppression is still not made following the cause–effect paradigm. Rather, such critical determination is essentially based on clinician's perception of the immunologic risk for rejection or infection. Therefore, precise and prospective immune monitoring to assess the antidonor alloimmune status of renal transplant recipients at different time points is highly required.[4,5]

The current evaluation of the immunologic risk in humans is rather poorly assessed as though only the presence of preformed circulating alloantibodies is determined, with the assumption that humoral allosensitization also illustrates the allospecific T-cell effector/memory immune response. However, it is well known that cellular memory may occur without humoral activation and are key factors in initiating and mediating allograft rejection.[6–9] As compared with their naive counterparts, alloreactive effector/memory T cells are long lived, have rapid recall effector function with reduced activation requirements, may also be influenced by heterologous immunity, and are notably less susceptible to immunosuppression.[10–13] Indeed, in clinical TX detection of highly alloreactive circulating memory/effector T cells with donor-antigen specificity, both before and after TX, using the highly sensitive interferon-γ (IFN-γ) enzyme-linked immunosorbent spot assay (Elispot), has been shown to discriminate patients at increased risk for T cell–mediated rejection and worse graft function evolution, even in the absence of humoral allosensitization.[14–21]

Importantly, decision-making regarding the type and amount of immunosuppression in renal TX is nowadays in a crossroad, as avoidance of calcineurin inhibitor (CNI)-related chronic nephrotoxicity and undesired side effects should

conciliate with an efficient abrogation of allograft rejection. In this line, although CNI drugs are associated with reduced incidence of acute rejection, the use of alternative immunosuppressants with other immunomodulatory effects, such as mammalian target of rapamycin inhibitors, has been suggested to not be sufficiently safe to control alloreactivity.[22]

Here we have prospectively assessed donor-specific (d-s) and non-d-s T-cell alloreactivity using the IFN-γ Elispot assay in a group of 60 consecutive kidney transplant patients allocated to receive a CNI-based or CNI-free immunosuppressive regimen, if the presence or absence of detectable antidonor alloreactive T-cell frequencies were detected before TX, respectively. The impact of d-s T-cell alloreactivity on kidney allograft function and on allograft damage in 6-month protocol biopsies was evaluated.

RESULTS

Patient distribution depending on their antidonor T-cell sensitization

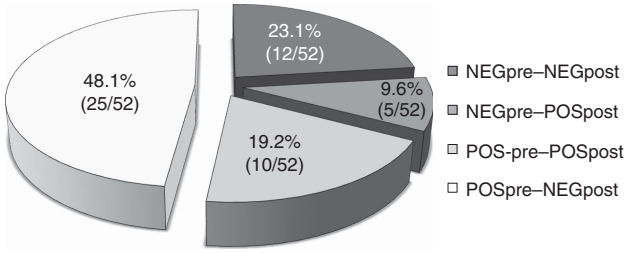
Before TX, 38/60 patients showed highly alloreactive circulating d-s T cells, whereas 22/60 did not. At 6 months, 52 patients could be evaluated for a second Elispot (35 alloreactive and 17 nonalloreactive). Although 37/52 (63.3%) patients displayed a negative d-s Elispot, 15/52 (28.8%) showed a positive test. As a result, 71.4% of pretransplant T cell–sensitized patients showed a negative 6-month d-s Elispot and 28.6% remained positive. Within the negative pretransplant Elispot, 70.6% remained negative but 29.4% became positive (Figure 1).

Among the 12 non-d-s alloreactive patients both before TX and at 6 months (negative pre-TX d-s IFN-γ Elispot–negative 6-month d-s IFN-γ Elispot (NEGpre–NEGpost)), 9 (75%) showed d-s hyporesponsiveness (DSH) and 3 (25%) were T-cell unreactive to both third-party (3P) and viral (cytomegalovirus and CEF (peptide stimuli containing cytomegalovirus, Epstein–Barr, and influenza virus lysates) stimuli (nonresponders). Conversely, within the 25 positive pre-TX d-s IFN-γ Elispot–NEGpost (POSpre–NEGpost) patients, only 9 (34%) were DSH and 16 (64%) were nonresponders ($P = 0.05$).

Immunosuppression allocation was done following the study protocol (Figure 2). At 6 months, 35 POSpre patients were on tacrolimus (TAC) + mofetil mycophenolate (MMF) + prednisone (PDN) and 17 NEGpre were on sirolimus (SRL) + MMF + PDN. After the 6-month Elispot and allograft biopsy, immunosuppression was modified per protocol. As a result, in 22/35 (63%) of the POSpre–NEGpost patients PDN were withdrawn, while 13 (37%) were maintained on TAC + MMF + PDN. Regarding the 17 NEGpre group, in 11 (65%) patients PDN and MMF were progressively withdrawn, and 6 (35%) patients were switched to TAC + MMF + PDN.

Main clinical outcome

As shown in Table 1, highly pretransplant T-cell alloreactive patients were comparable to nonalloreactive patients regarding the most important demographic characteristics.



	NEGpre 22/60 (37.7%)	POSpre 38/60 (63.3%)
NDpostTX	5/22	3/38
NEGpostTX	12/17 (70.5%)	25/35 (71.4%)
POSpostTX	5/17 (29.4%)	10/35 (28.5%)

Figure 1 | Distribution of patients depending on the donor-specific (d-s) T-cell alloimmune response. Twenty-five out of 35 (71.4%) of pretransplant T cell–sensitized patients (positive pre-TX d-s IFN-γ Elispot (POSpre)) showed the absence of antidonor T-cell alloresponses (POSpre–negative 6-month d-s IFN-γ Elispot (NEGpost)), but 10/35 (28.6%) still showed evidence of antidonor T-cell alloreactivity (POSpre–positive 6-month d-s IFN-γ Elispot (POSpost)). Within the 17 patients without d-s T-cell alloreactivity before TX, 12 (70.6%) remained negative (negative pre-TX d-s IFN-γ Elispot (NEGpre)–NEGpost), but 5 (29.4%) displayed antidonor T-cell alloreactivity (NEGpre–POSpost). Elispot, enzyme-linked immunosorbent spot assay; IFN-γ, interferon-γ; ND, not done; TX, transplantation.

As shown in Table 2, pretransplant T-cell alloreactive patients (positive pre-TX d-s IFN-γ Elispot (POSpre)) showed significantly higher incidence of opportunistic infections than NEGpre individuals, and this was especially evident among unresponder patients at 6 months. Incidence of peritransplant urological-related complications was comparable in both groups, although two NEGpre patients were switched to TAC because of unresolved urinary leakage (one lost the graft). Two POSpre patients passed away due to *Pneumocystis jiroveci* pneumonia and lung cancer at months 4 and 5, respectively. One-year graft and patient survival were similar between both groups.

The incidence of biopsy-proven acute rejection in the study was very low, i.e., 5% (3/60). All events that occurred in the NEGpre group occurred at days 15 (humoral), 35, and 43 (cellular) after TX, and were all successfully treated.

Six-month histological damage. Six-month protocol biopsies could be done in 46/53 (87%) patients. Acute T cell–mediated subclinical rejection (TCSCR) was observed in 11/46 (24%) patients, and 5/46 (6.5%) displayed histological patterns of antibody-mediated rejection. Chronic histological damage (IF/TA > I) was observed in 11/46 (23.9%) patients (76.1% IF/TA ⩽ I, 15.3% IF/TA = II, and 8.7% IF/TA = III). One patient showed evidence of BK virus nephropathy (confirmed by BK viremia). Neither the presence of SCR nor evidence of IF/TA was associated with the pretransplant Elispot.