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INVITED COMMENTARY

Drug-minimization or tolerance-promoting strategies in human kidney transplantation: is Campath-1H the way to follow?

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Kidney transplantation is the therapy of choice for most patients with end-stage renal diseases. Although current immunosuppressive strategies yield excellent 1-year graft and patient survival and low-acute rejection rates (commonly <20%), long-term outcomes still need to be improved. Indeed, chronic allograft nephropathy leading to progressive graft dysfunction and loss is not prevented and may be due, at least in part, to chronic calcineurin inhibitor (CNI) use [1–3]. Furthermore, immunosuppressive drugs are associated over the long term with relatively high rates of complications because of their potential adverse renal, cardiovascular, and metabolic side-effects [4–7]. Thus, to improve outcomes, new therapeutic strategies must be investigated that may allow minimization of chronic immunosuppression, while achieving long-term graft acceptance with normal organ function. Despite its great difficulties and relatively slow progress over the years, the ultimate goal in transplantation remains the induction of operational tolerance, which is defined by a state of durable donor-specific unresponsiveness, in the absence of immunosuppressive drug therapy.

T cells are crucial in the initiation and the coordination of the rejection response and, to achieve immunological unresponsiveness, it is important to deplete or minimize the peripheral alloreactive effector T-cell pool [8]. Various strategies that target T-cell activation, expansion and effector functions have been explored in experimental animal models to promote peripheral tolerance, and some such as costimulatory blockade or lymphocyte-depletion induction therapies are now also being evaluated in clinical trials [9–11].

It is known that the events related to organ implantation and the resulting ischemia-reperfusion injury ('danger' signals) will potentiate alloantigen presentation and the activation of the immune system [12]. Given at the time of transplantation, induction strategies using cell-depleting approaches can result in a profound reduction of circulating lymphocytes capable of mounting an alloresponse at a time when the allograft is already susceptible to inflammatory damage. Lymphocytes will gradually repopulate the recipient weeks to months later, i.e. at a time when the innate immune response has resumed and the allograft is more quiescent [13–15]. Thus, initial T-cell depletion reduces the risks of early acute rejection episodes and it may help promoting the induction of tolerance.

Depletion strategies using anti-T-cell monoclonal antibodies (mAbs) have been extensively studied in nonhuman primate (NHP) transplantation models, alone or in

combination with other immunomodulatory drugs. Encouraging results in these animal models paved the way to the clinical trials by using alemtuzumab (Campath-1H) in kidney transplant recipients as a means of minimizing the immunosuppression [9,16,17]. Campath-1H is a humanized CD52-specific mAb that profoundly depletes mature T cells, and to a lesser extent B cells, monocytes, macrophages and natural killer (NK) cells from the peripheral blood and lymph nodes.

In this issue of *Transplant International*, Barth *et al.* [18] report the use of Campath-1H in an approach to minimize the maintenance immunosuppression in low-risk kidney transplant recipients of primary cadaveric and living donor allografts. The short-term results were previously reported [19] and these are now the 3-year follow-up outcomes. Although they have studied a relatively small cohort (29 patients), these extended results contribute, together with previous reports, to a better understanding of the mechanisms of action of Campath-1H and they also highlight the advantages and limitations of drug-minimization approaches based on the lymphocyte-depletion strategies.

Previous extensive studies in NHP models using anti-T-cell agents such as rabbit ATG or the anti-CD3-immunotoxin had shown that, despite profound peritransplant T-cell depletion, consistent transplantation tolerance was not achieved, as most treated animals eventually lost their grafts through chronic rejection [20–22]. Similar results were reported in a selected group of seven human kidney transplant recipients from living donors, who received Campath-1H in the immediate pre- and post-transplant period. Indeed, all patients experienced reversible acute rejections and some maintenance immunosuppression (sirolimus monotherapy) had to be introduced after the treatment of the episode of rejection [23]. Taken together, these earlier results pointed out that although anti-T-cell mAbs greatly reduced the requirements for maintenance immunosuppression, they could not induce true tolerance. Kirk's group subsequently investigated the combination of polyclonal rabbit antithymocyte globulin with sirolimus in clinical kidney transplantation [24].

As reported in this issue, Knechtle's group chose to use sirolimus in combination with Campath-1H induction in human kidney transplant recipients. This was a logical choice as long-term immunosuppression based on sirolimus, besides having a positive impact on kidney function when compared with cyclosporine [25], may add a beneficial effect as this mTOR inhibitor is thought to favor the induction of peripheral transplantation tolerance. Sirolimus was shown experimentally to facilitate peripheral deletion of effector alloreactive T cells by promoting activation-induced cell death, leaving a small pool of residual alloreactive T cells, which could be regulated by $CD4^+CD25^+$ regulatory T cells (Tregs) [26,27]. Furthermore, recent data have demonstrated that sirolimus can selectively expand Tregs *in vitro* and *in vivo* [28,29], while CNIs appear to have an inhibitory effect of Tregs expansion and function [30]. Campath-1H induction combined with sirolimus from day 1 resulted in excellent graft (96%) and patient (100%) survival at 3 years, with good graft function, and 12 of 28 patients (43%) could remain on the original sirolimus monotherapy. Moreover, 67% of patients were on steroid-free immunosuppression at 3 years. However, Barth *et al.* report a relatively high rate of acute rejection (46%), characterized as humoral rejection with C4d positivity in more than half of these episodes. Many of these humoral rejection episodes occurred early, within 1 month after transplantation, despite the initial profound T- and B-cell depletion in the peripheral blood. The addition of thymoglobulin at induction in four patients did not appear to modify this trend. In view of this unexpected rate and type (antibody-mediated) of acute rejection, one can wonder if the small amount of remaining T- and B cells were sufficient to mount an alloimmune response, or whether other effector cells should have been targeted as well.

Previous data have suggested that the rejection episodes seen after Campath-1H induction therapy may differ from that seen under conventional CNI-based immunosuppressive protocols [15,23,31]. In accordance to previous studies, Barth *et al.* report that peripheral T-cell depletion in their series was near complete during the first year after transplantation (77% depletion of $CD3^+$ T cells at 12 months) and was sustained with over 50% depletion still at 3 years. B-cell depletion in the periphery was also very effective in the first months, but the recovery started within 3–6 months with reconstitution to baseline values by 2 years. Furthermore, it is known that most plasma cells are not depleted by Campath-1H. Thus, the B-cell lineage may need to be more efficiently targeted when using an immunosuppressive approach combining Campath-1H with sirolimus.

Perhaps importantly, it has also been shown that Campath-1H is less effective at depleting monocytes/macrophages and NK cells. When using Campath-1H alone in kidney transplant recipients, Kirk *et al.* [23] described early rejection episodes with predominantly monocytic infiltrates and only rare T cells, coinciding with peripheral monocyte repopulation. These monocytes might have been activated following the ischemia-reperfusion injury and could have mediated rejection in part by secreting cytokines that in turn recruited residual effector T cells or NK cells. To corroborate this hypothesis, the authors found a high level of HLA-DR expression and elevated transcript levels for TNF-α and interferon-γ in the rejecting allografts. However, combining lymphocyte-depletion with deoxyspergualin (DSG), a drug which inhibits monocytes/macrophages, did not induce tolerance clinically, as

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all patients developed rejection episodes that were similar in timing and histology to that seen in patients treated with Campath-1H alone [15]. These disappointing results in humans, therefore, did not confirm previous promising data in NHP models [32]. By efficiently depleting most T cells, Campath-1H might have also affected the homeostasis of Tregs. Indeed, the induction and maintenance of peripheral immune tolerance is an equilibrium between pathogenic and regulatory mechanisms and, besides inhibiting the effector function of $CD4^+$, $CD8^+$ T cells and B cells, Tregs were also shown to exert direct suppressive effects on monocytes/macrophages [33,34].

Although anti-T-cell antibodies can deplete nearly all circulating peripheral T cells (>99% depletion), they are slightly less efficient on cells that have homed to peripheral tissues and lymph nodes. Furthermore, it appears that effector memory T cells, that differ from naïve T cells by their activation requirements and *in vivo* trafficking patterns, are more resistant to antibody-mediated T-cell depletion [31]. Thus, this small pool of 'depletion-resistant T cells' could proliferate and contribute to the rejection process associated with monocytes, as well as provide help to the repopulating B cells [35]. In fact, pre-existing memory T cells are now considered to be a major hurdle to the induction of tolerance in adult human transplant recipients [35–40]. The proportion of memory T cells in the alloreactive human T cell repertoire may also explain the discrepancy between encouraging preclinical transplantation studies (using animals living in protected 'cleaner' environments) and somewhat disappointing tolerance-promoting clinical trials. However, the relative sparing of memory T cells may be beneficial in terms of infection control, as the use of Campath-1H was not associated with a higher incidence of infectious complications in most series.

Overall, in recent years, the Campath-1H induction studies have indicated that this potent agent is well tolerated, but it does not induce clinical transplantation tolerance. Various patterns of rejection (e.g. alloantibody-mediated or monocytic) can be observed if monotherapy with sirolimus or DSG is used as only maintenance immunosuppression following the Campath-1H induction. As discussed by Barth *et al.*, the incorporation of a CNI following intense lymphocyte depletion appears to be needed to prevent early acute rejection [18,31]. An important question will now be to define the optimal maintenance drug regimen to be used following the Campath-1H induction [18,19,41–43], if the main objective is to achieve long-term drug-minimization in solid organ transplant recipients. Here, it should be emphasized that continued long-term follow-up and monitoring will be required in protocols designed to minimize the immunosuppressive drugs. For example, careful prospective assessment of long-term allograft function and histology, as well as immunologic monitoring of class I and class II alloantibodies and of donor-reactive T-cell responses, all appear mandatory in the upcoming years. Cell-depleting induction protocols with agents such as Campath-1H may or may not allow for long-term drug-minimization in the majority of transplant recipients. The report by Barth *et al.* in this issue is interesting and brings us a step further. However, there is still a long way to go before we will have all the answers that we need in order to consistently optimize the long-term outcomes of organ transplant recipients.

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