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Prolonged lymphocyte depletion by single-dose rabbit anti-thymocyte globulin and alemtuzumab in kidney transplantation

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ABSTRACT

Although antibody induction has gained in popularity, two agents are rarely combined. We retrospectively analyzed peripheral lymphocyte phenotypes of renal transplant recipients who received induction therapy with a different antibody/combination: alemtuzumab(C1H), Thymoglobulin(rATG), daclizumab(Dac), rATG + C1H, and rATG + Dac. CD4+ T-cells were suppressed by C1H and rATG + C1H, as well as by rATG and rATG + Dac but to a lesser extent. The effect lasted for 3 years at around 40% of baseline values. CD8+ T-cells showed a similar trend but had a more rapid recovery to baseline. CD19+ B-cells were effectively suppressed for 2 months by C1H and rATG + C1H, and abruptly returned to baseline afterwards; suppression by rATG(7 doses) was modest but lasted longer. A higher proportion of CD56 + CD16+ Natural Killer cells in C1H treated patients suggested a relatively spared effect of C1H on this cell type. Low CD25+ T-cells by 5-dose Dac returned to baseline around 6 months, whereas rATG + C1H and rATG + Dac showed persistent effect. CD4 + CD25hi T-cells were suppressed by both rATG + C1H and rATG + Dac, but the initial proportion of CD4 + CD25hi T-cells among CD4+ T-cells and CD4 + CD25hi/CD4 + CD25lo ratio were significantly higher in rATG + C1H. Overall, with extensive and persistent lymphocyte suppression by a simple administration of agents, single-dose rATG + C1H induction can be an alternative in renal transplantation.

1. Introduction

Antibody induction has been widely used in kidney transplantation and its application expands as steroid- or calcineurin inhibitor (CNI)-sparing protocols are more commonly employed [1]. Anti-interleukin-2 receptor antibodies (i.e. basiliximab and daclizumab [Dac]) decrease the incidence of acute rejection after kidney transplantation but their efficacy in high-risk patient population (e.g. black race, highly sensitized, receiving marginal donor kidneys) may be limited especially when maintenance immunosuppression is reduced [2]. As compared with polyclonal antithymocyte globulin (e.g. Thymoglobulin [rATG], Atgam, ATG-Fresenius), a humanized anti-CD52 monoclonal antibody alemtuzumab (Campath-1H [C1H]) has some advantages; convenient one- or two-dose infusion of C1H effectively suppresses peripheral lymphocytes for a prolonged period of time. However, C1H induction may be associated with increased incidence of monocyte-mediated and antibody-mediated rejection [3 10].

Although combinations of CNIs, antimetabolites, mTOR inhibitors, or corticosteroids are the mainstay of maintenance immunosuppressive therapy, two or more antibodies are rarely combined for induction therapy [11 15]. We recently demonstrated that combined use of rATG and Dac in both kidney and kidney-pancreas transplantation effectively suppress activated T-lymphocytes in the peripheral blood [14]. This strategy has also been described by others using dual induction of rATG and basiliximab [12]. To further explore the possibility of antibody combination therapy, we have conducted a prospective randomized trial comparing a new antibody combination single-dose rATG and single-dose C1H and our current standard combination 3-dose rATG and 2-dose Dac (submitted). The new combination can theoretically achieve the beneficial effects of both agents, i.e. reduced

Abbreviations: C1H, alemtuzumab (Campath-1H); rATG, antithymocyte globulin (Thymoglobulin); Dac, daclizumab; Ritux, rituximab; CNI, calcineurin inhibitor; mTOR, mammalian target of rapamycin; MMF, mycophenolate mofetil; EC-MPS, enteric coated mycophenolate sodium; AR, acute rejection; BAFF, B-cell activating factor; FOXP3, forkhead box P3.

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ischemia/reperfusion injury and profound and prolonged T- and B-cell depletion with low incidence of acute rejection.

2. Objective

This paper specifically discusses lymphocyte depletion and reconstitution of the peripheral blood in kidney transplant recipients who received different antibody induction agent(s). Comparisons of clinical outcome data (e.g. acute rejection, infections, graft and patient survival) are described elsewhere [16, 17, submitted] and are beyond the scope of this manuscript, and therefore are not reported.

3. Material and methods

3.1. Patients and protocols

We retrospectively analyzed renal transplant recipients in three prospective randomized clinical trials [16,17]. Between September 2002 and October 2006, adult, primary living-donor (NCT00681343) or deceased-donor (NCT00685061) renal transplant recipients entered randomized studies comparing 3 antibody induction therapies: C1H, rATG, and Dac. Between February 2006 and October 2007, adult, primary recipients of either a deceased- or living-donor renal transplant entered another study (NCT01172418) comparing two antibody combinations: rATG + C1H vs. rATG + Dac. The center institutional review board approved the studies, and all patients gave written informed consent prior to enrollment. The induction and maintenance immunosuppresive protocols of the study groups are summarized in Table 1. Patients who received any extra doses of rATG, a different antibody (i.e. rituximab), or plasmapheresis for delayed graft function, early acute rejection, or other clinical indications were excluded from the primary analysis. The data of patients who developed acute rejection and received high-dose corticosteroids or antibody treatment were excluded only after the rejection event to exclude the effects of augmented immunosuppression.

3.2. Flow cytometric analysis

We measured cell surface markers of peripheral white blood cells at predetermined time points using a FACSCalibur ow cytometer and CellQuest software (BD Biosciences, San Jose, CA) from pretransplant (baseline) to 3 years posttranspalnt. The cells were stained as previously described [18] using direct immuno uorescence staining of cell surface antigens in unseparated blood with three- or four-color cocktails that included monoclonal antibodies to CD45, CD2, CD3, CD4, CD8, CD19, CD25, CD34, CD52, CD56/CD16, and HLA-DR epitopes (BD). The absolute numbers of cells expressing a particular subset phenotype were determined by multiplying the respective percentages obtained by ow cytometry by absolute white cell counts from hematologic whole blood measurements. Monocyte counts were provided by hematologic measurements. Mean values (pooling the data across patients) were obtained for measurements categorized according to the groups and time of measurement.

3.3. Statistical analysis

We computed data using JMP 7.0.2 (SAS Institute, Inc., Cary, NC) and reported results in the tables as mean ± standard deviation and data in the figures using mean ± standard error of the mean as error bars. One-way analysis of variance, a Dunnett's method (pretransplant as a control), and a Tukey-Kramer's honestly significant difference (HSD) test (for multiple comparisons) were used to compare means.

4. Results

4.1. Patient demographics

For C1H, rATG, and Dac comparison, 38 living-donor renal recipients and 90 deceased-donor renal recipients entered the prospective randomized studies. After removing patients with extra immunosuppression as described in the Methods section, 116 were included in the analyses. The study comparing rATG + C1H vs. rATG + Dac enrolled 150 patients; of those, data from 120 patients were eligible and available for the analyses. Distributions of the baseline demographics are listed in Table 2. The study population consisted of a diversity of patients, including 37% Hispanics and 23% African-Americans; 30% were female. The majority of renal transplants were performed from deceased donors (72%). Cold ischemia time was relatively long with 82% of deceased donor kidneys being preserved for more than 24 h; pulsatile machine perfusion was used for all deceased donor cases [19].

4.2. T cell depletion and reconstitution

The mean T-cell counts reached a nadir during the first week in all groups (Fig. 1A). There was a noticeable initial steep decline of CD3+ T cells from the pretransplant levels in both C1H and rATG treated patients. The fall in the mean CD3+ T-cell count was most dramatic in C1H-based induction protocols (i.e. 2 doses of C1H and 1 dose of C1H plus 1 dose of rATG) with a nadir approaching total peripheral depletion of CD3+ T cells; CD3+ counts remained fewer than 100 per microliter (or less than 10% of pretransplant values) for more than 2 months in these two groups. Surprisingly, both C1H based protocols maintained 50% CD3+ depletion for 3 years only with low-dose tacrolimus and mycophenolate maintenance therapy. In contrast, the effect of rATG on T-cell depletion was significantly shorter than C1H. Although a nadir CD3+ value reached 1% of pretransplant value at 1 week after 7 doses of rATG treatment, it returned to 10% of baseline within 1 month and increased to 50% range by 6 months. The effect of 3-dose rATG combined with 2-dose Dac was similar but with even quicker recovery, reaching 30% of pretransplant value at 1 month.

When CD4+ T cells and CD8+ T cells are evaluated separately (Figs. 1B, C, 2A and B), both C1H-based and rATG-based induction protocols exerted more profound depletion of CD4+ T cells than CD8+ T cells, leaving CD4+/CD8+ ratio lower than 1 for the initial few months. CD4+ frequency in peripheral leukocyte (CD45+) also declined dramatically after treatment with depleting antibodies (Table S1). C1H and C1H + rATG induction depleted CD4+ T cells to fewer than 1% of pretransplant values and maintained lower than 10% for 5 months. Although initial recovery from nadir values (1% and 6% in the rATG and rATG + Dac groups, respectively) is quicker, prolonged depletion similar to that seen with C1H was observed with rATG based induction for 3 years (roughly 40% of pretransplant values). C1H and rATG + C1H protocols depleted CD8+ T cells effectively for the first

Table 1
Immunosuppressive protocols.

	rATG + C1H	rATG + Dac	C1H	rATG	Dac
Induction					
rATG	1 mg/kg × 1	1 mg/kg × 3		1 mg/kg × 7	
C1H	0.3 mg/kg × 1		0.3 mg/kg × 2		
Dac		1 mg/kg × 2			1 mg/kg × 5
Maintenance					
Tacrolimus	4 7 ng/mL	4 7 ng/mL	4 7 ng/mL	8 10 ng/mL	8 10 ng/mL
MMF†			0.5 g twice daily	1 g twice daily	1 g twice daily
EC-MPA†	360 mg twice daily	720 mg twice daily			
Corticosteroids	1 week only	1 week only	1 week only	Maintenance	Maintenance

* Indicates targeted trough levels. † indicates scheduled doses of MMF or EC-MPA. rATG: rabbit antithymocyte globulin (Thymoglobulin ®), C1H: alemtuzumab (Campath-1H®), Dac: daclizumab (Zenapax®), MMF: mycophenolate mofetil (CellCept®), EC-MPA: enteric-coated mycophenolic acid (Myfortic®).