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Prophylactic use of the IL-2 receptor-specific monoclonal antibody LO-Tact-1 with cyclosporin A and steroids in renal transplantation

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Abstract. LO-Tact-1 is a rat anti-human monoclonal antibody which is directed to the 55-kD$_a$ α-chain of the interleukin 2 (IL2) receptor. We conducted a pilot trial in 15 first-time cadaveric renal transplant patients undergoing for immunosuppression a 14-day course of LO-Tact-1 (10 mg IV daily) together with cyclosporine, low dose steroids (0.5 mg/kg) and azathioprine. Results showed a good immunosuppressive effect, as measured by the similar incidence of acute rejection episodes (0.6 per patient) when compared with 20 patients treated during the same period with our standard quadruple prophylactic combination with higher initial doses of steroids (2 mg/kg) and antilymphocyte globulin (ALG) instead of LO-Tact-1 (0.4 per patient). At 2 years post-transplant, graft survival was 93%, and only 1 patient lost his kidney by rejection. No local or general adverse effect of antibody administration was encountered, and haematological changes remained of minor importance. Local bacterial infection was observed in 3 patients, but viral diseases (including cytomegalovirus, CMV) remained exceptional. In contrast, severe clinical CMV infections occurred in 3 patients (15%) treated by ALG. Nine of 15 patients developed rat-specific antibodies, but only 4 before the completion of LO-Tact-1 treatment, without any correlation with the further development of acute rejection. Patients who suffered rejection had lower LO-Tact-1 levels and higher soluble IL2 receptor levels during the period of infusion, suggesting the crucial importance of pharmacokinetic monitoring to adjust individual doses.

Key words: Interleukin 2 receptor – Monoclonal antibodies – Renal graft rejection

The prophylactic use of polyclonal anti-lymphocyte (ALG) or anti-thymocyte (ATG) globulins following renal transplantation has become one of the most effective and safe strategies for managing renal transplant recipients during the immediate postoperative period [4, 6]. However, the more recent availability of monoclonal antibodies directed against targets on the T-lymphocyte membrane has enabled the clinical use of highly selective immunosuppression. Indeed, OKT3, a monoclonal antibody directed to the invariant CD3 component of the T-cell-receptor complex, is routinely employed, with a remarkable effect in preventing early rejection, but with the disadvantages of inducing severe and even life-threatening first-use reactions and being associated with an increased number of opportunistic infections [3, 13]. A more specific and less toxic kind of immunosuppression is to target only cells involved in the rejecting process which are expressing activation antigens. Among these antigens, interleukin 2 (IL2) receptor plays a crucial role by controlling the proliferative expansion of T lymphocytes. Several monoclonal antibodies specific for the low-affinity IL2 receptor have been produced and have demonstrated their ability to inhibit IL2 binding to its receptor. They have been shown to be effective in the prophylaxis of allograft rejection both in animal models [5] and in human transplantation [6, 7, 9]. We report herein the results of a pilot study conducted in 15 first time cadaver kidney transplants, treated for the prophylaxis of rejection with LO-Tact-1, a rat immunoglobulin (IgG2b) directed to the 55D$_a$ α-chain of the IL2 receptor, in combination with cyclosporine (CsA), low dose corticosteroids and azathioprine (AZA).

Materials and methods

Patient population. From May through August 1989, 15 study patients were elicited to receive a quadruple prophylactic regimen including LO-Tact-1 following their first renal transplantation. All patients who were transplanted in our unit during the week (from Monday to Wednesday) were included, in order to make easier the pre- and immediate post-transplant monitoring. During the same period, 20 patients who received their graft at the weekend (Saturday and Sunday) constitued the control group and received our

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Table 1. Main patient and transplantation characteristics

	LO-Tact-1 ($n = 15$)	Control ($n = 20$)
Mean age (range)	49.5 y (35–63)	40.2 (21–65)
Sex ratio (male/female)	11/4	12/8
Immunized (PRA > 80%)	8 (3)	8 (4)
Mean A-B mismatching	2.4 ± 0.2	2.4 ± 0.2
Mean DR mismatching	1.3 ± 0.2	0.9 ± 0.1
Cold ischaemia time (h)	31 ± 8.1	32 ± 7.5
Initial non-function	7 (47%)	11 (55%)

PRA, plasma renin activity

standard quadruple prophylactic immunosuppressive regimen with ALG. Although this pilot study was not randomized, no attempt was made to select the patients who received the monoclonal antibody, except that second transplants were excluded. The background characteristics of the patients and transplantation data are summarized in Table 1. Except for the mean age which was significantly higher in the study patients, there was no statistically significant difference between both groups with respect to the pre- and peritransplant variables.

Immunosuppressive regimens. Patients from the study group received LO-Tact-1 IV 10 mg daily for the first 14 days posttransplant. LO-Tact-1 is a rat anti-human monoclonal antibody of the IgG-2b isotype whic is directed to the 55-D_a α-chain of the IL2 receptor. It was developed at the Experimental Immunology Unit of the University of Louvain, Medical School, Brussels, Belgium [15]. It was produced in vivo from ascitic fluids of LOU/C·IgK1b-OKA rats. Many carefully controlled purifications were performed to ensure the purity of the antibody, as well as to avoid harmful contaminants [10]. LO-Tact-1 competitively inhibits the high affinity binding of iodine-125 IL2 to activated T lymphocytes. A 50% inhibition of radiolabelled IL2 binding is observed at a concentration of 8×10^{-9} *M* LO-Tact-1. The other immunosuppressive drugs included corticosteroids, a single bolus of 2 mg/kg methylprednisolone on day 0, then oral prednisone 0.5 mg/kg daily from day 1 to day 14, with subsequent doses being progressively reduced to a baseline of 10 mg/day at 1 month posttransplant. CsA was given IV on day 0 (4 mg/kg), then orally at 8 mg/kg, with the dose being further adjusted to whole blood trough levels (TDX; Abbott) and to the clinical events if acute nephrotoxic episodes occurred. AZA was introduced on day 45 at an initial dose of 1 mg/kg daily, eventually being reduced when the white blood cell (WBC) count had decreased under 4000/mm³.

Control patients received instead of monoclonal antibody a prophylactic course of polyclonal ALG (Lymphoglobuline; Merieux) 15 ml/day IV from day 1 to day 14 and a higher initial oral dose of prednisone: 2 mg/kg from day 1, reduced to 1 mg/kg at day 14, then to the baseline dose of 10 mg/day at 2 months posttransplant.

Histologically confirmed rejections were treated in both groups by IV methylprednisolone bolus for 6 days (10 mg/kg on day 1, then 5, 4, 3, 2 and 1 mg/kg). When rejections occurred later than the 14-day period of rejection prophylaxis, patients received an additional 7-day course of polyclonal ATG (Thymoglobuline; Merieux) 15 ml/day. Rejections that were resistand to a first anti-rejection therapy were treated by OKT3 monoclonal antibody 5 mg/day IV for 7 days.

Immunological monitoring. Serum samples were obtained preoperatively and every 2 days postoperatively until the 1 month after transplantation from patients receiving LO-Tact-1. The trough levels of LO-Tact-1 were retrospectively measured by enzyme-linked immunosorbent assay (ELISA), as described elsewhere [10]. LO-Tact-1-specific IgG and IgM antibodies were detected by another ELISA [10]. The serum concentration of the soluble IL2 receptor was measured twice weekly in patients of both groups. Whole blood samples were also collected in heparinized tubes for immunofluorescence flow cytometric analysis of different subsets of peripheral blood lymphocytes.

Statistical analysis. Actuarial graft survival and rejection probability curves were calculated by the Kaplan-Meier method and compared by log-rank test. The χ^2 and Student's *t*-test were used for other comparisons when appropriate.

Results

Graft and patient survival

No patient died who had received the quadruple therapy with LO-Tact-1 for the induction protocol. Among the 20 control ALG patients, 1 died at 4 months posttransplant from severe cytomegalovirus (CMV) infection. In the LO-Tact-1 group, the actuarial graft survival was 100% at 1 year and 93% at 2 years (Fig. 1). The only graft loss at 21 months posttransplant was due to chronic rejection. In the control group treated with ALG, graft survival was similar, 85% at 1 and 2 years. The causes of 2 graft losses in this group other than patient death were 1 immediate hyperacute rejection and 1 chronic rejection at 5 months.

Incidence of rejection and graft function

During the period of administration of LO-Tact-1, 2 patients had histologically proven acute rejection (13.3%), this incidence being similar in control patients (2/20, 10%). As shown in Fig. 2, the total number of rejections

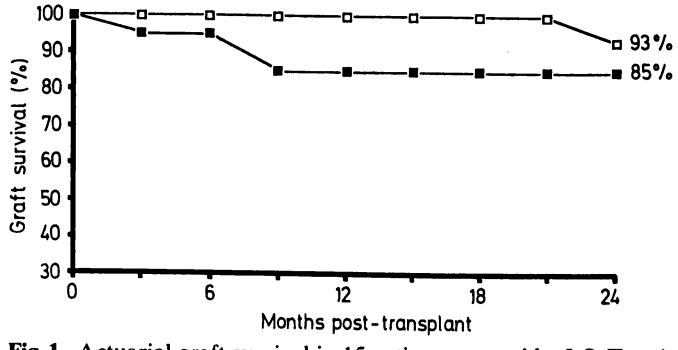

Fig. 1. Actuarial graft survival in 15 patients treated by LO-Tact-1 *(open squares)* and 20 control patients treated by antilymphocyte globulin (ALG; *(filled squares)*

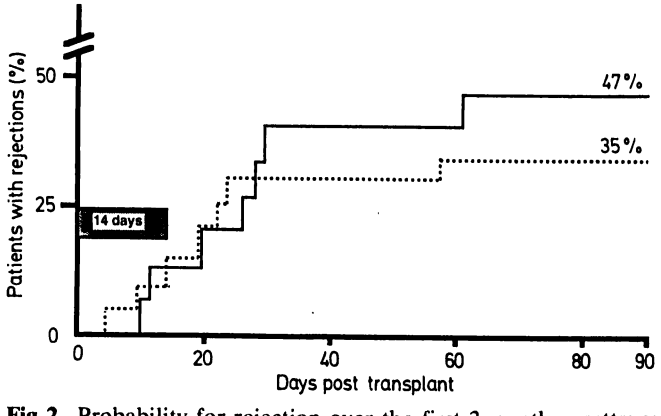

Fig. 2. Probability for rejection over the first 3 months posttransplant. *Solid line*, 15 LO-Tact-1 patients; *dashed line*, 20 control ALG patients

(9/15, 0.6 ± 0.19 per patient) recorded during the first 3 months posttransplant in 7 of the 15 study patients (47%) was comparable but slightly higher than in the control group: 8 rejections in 7 (0.4 ± 0.13 per patient) of the 20 patients (35%) treated by ALG. The time to the first rejection was also comparable in LO-Tact-1 patients (26.4 + / − 6.5 days) and in the control group (21.4 ± 6.3 days).

All acute rejection episodes were reversible in study patients, but 2 required a second anti-rejection treatment with OKT3. Chronic rejection was histologically documented in 3 patients at 2 years posttransplant. Similarly, one control patient of 7 experiencing rejection in this group required a second course with OKT3, but at 2 years, the incidence of chronic rejection was slightly higher than in the study patients: 6 of 18 patients who survived more than 3 months. At 2 years posttransplant, there was no difference between mean serum creatinine levels between study patients with functional grafts ($n = 14$; 147 ± 14 μmol/l) and control patients with functional kidneys ($n = 17$; 171 ± 26 μmol/l).

Infectious complications and tolerance

Only minor infections not directly related to immunosuppression were observed in LO-Tact-1 patients (2 urinary tract infections, and 2 local wound infections). Viral episodes remained exceptional in our group (1 local herpes simplex in a patient treated by OKT3). Comparatively, 3 CMV infections (including 1 lethal one) occurred in patients treated by ALG, this incidence of 15% being usual for patients treated by such a quadruple immunosuppressive regimen [6]. Considering the small number of patients entered in this study, no statistical conclusion was demonstrable.

After transplantation, a profound lymphopenia was observed in patients treated by polyclonal ALG, which persisted to the end of the 1 month posttransplant (total lymphocyte count per mm^3 2600 ± 460, 450 ± 120, 560 ± 220 and 900 ± 210 on days 0, 7, 14 and 28, respectively). In patients treated with LO-Tact-1, the total lymphocyte count dropped only moderately and remained significantly high ($P < 0.05$) from day 7 to day 28 (1900 ± 150, 1050 ± 100, 1530 ± 160 and 1540 ± 260 on days 0, 7, 14 and 28, respectively). Similarly, dramatic drops in CD3+, CD4+ and CD8 cell counts were observed in ALG patients, whereas only mild and transient decreases were observed during LO-Tact-1 treatment.

During the administration of LO-Tact-1, no major complication requiring the discontinuation of treatment was observed. In two patients, mild febrile episodes occurred, but with no evident relationship to the LO-Tact-1 infusion.

Immunological monitoring

Since the values of the LO-Tact-1 levels and information about the development of rat-specific antibodies were only retrospectively obtained, no attempt was made to adjust the dose to the trough levels or discontinuing treatment in immunized patients.

Nine study patients (60%) developed LO-Tact-1-specific IgG antibodies. In 4 (26.7%), these antibodies were detected during the 14-day period of LO-Tact-1 administration. There was no correlation between rejection and immunization: 3 of the 7 patients who experienced rejection developed LO-Tact-1-specific IgG (43%). IgM LO-Tact-1-specific IgM antibodies were detected in all patients before day 14, and as earlier as day 4 in 2 patients.

The mean trough levels of LO-Tact-1 increased progressively from 1.2 ± 1 μg/ml on day 2 to 2.2 ± 1 on day 4 and then remained stable throughout the LO-Tact-1 treatment (2.9 ± 2.5 μg/ml at day 14). There was no difference between the LO-Tact-1 mean levels in patients who developed LO-Tact-1-specific IgG and IgM antibodies and mean levels in patients developing only IgM. Importantly, there was a negative correlation between LO-Tact-1 trough levels and rejection: the group of 7 rejecting patients had significantly ($P < 0.05$) lower mean LO-Tact-1 levels from day 2 to day 7 (0.3, 1.3 and 1.2 μg/ml at days 2, 4 and 7, respectively) than patients who suffered no rejection episode during the 3-month posttransplant period (1.7, 2.7 and 2.4 μg/ml).

The plasma levels of the soluble IL2 receptor were significantly ($P < 0.05$) lower in LO-Tact-1 patients from day 2 (79 ± 16 pmol/ml) to day 17 (132 ± 35 pmol/ml) than in ALG patients (183 ± 24 and 240 ± 41 on days 2 and 17, respectively). There was no significant correlation between the soluble IL2 receptor plasma levels, anti-IgG immunization and the rejection episodes in patients treated with LO-Tact-1. Finally, the group of 7 patients who were treated for acute rejection had significantly lower soluble IL2 receptor levels from day 7 to day 14 posttransplant.

Discussion

The results of our pilot study conducted in 15 first-time cadaveric transplant patients suggest that an immunosuppressive prophylactic regimen including LO-Tact-1, a rat IL2 receptor-specific monoclonal antibody, is highly effective in preventing rejection. This effect is roughly similar to that obtained with a powerful quadruple prophylactic combination with polyclonal horse ALG, although we employed a fourfold lower (0.5 versus 2 mg/kg) initial dose of steroids. Moreover, despite the prolonged (14 days) serotherapy, we did not observed a significant number of opportunistic infections, and changes in the WBC and T-cell subsets remained transient and of minor importance. The local and general tolerance was excellent during LO-Tact-1 infusions. More than 2 years posttransplant, 12 patients (80%) had a normal renal function, and only 1 graft was lost by chronic rejection. Our results are similar to those reported by Soulillou et al. [12] with a different rat P55-specific monoclonal antibody (33 b3.1), utilized in a different immunosuppressive regimen: higher initial doses of steroids, delayed introduction of CsA, and AZA given from day 1. Using another mouse receptor-specific IL2 monoclonal antibody (anti-Tac) in addition to a triple drug regimen (steroids, low dose CsA and AZA), Kirkman et al. [9] reported the efficacy of their antibody in preventing al-

lograft rejection, but with a higher incidence of opportunistic infections, including lethal CMV infections, probably related to excessive immunosuppression. Both these large, randomized studies and our pilot trial are dealing with a clear effect of P55-specific monoclonal antibodies in the prophylaxis of rejection after renal transplantation. However, it should be pointed out that if monoclonal antibodies are able to produce comparable clinical results to old-fashioned drugs such as ALG, they do not seem to have a superior effect, and there was no clinical support for the in vitro demonstrated synergy between CsA and IL2-specific antibodies [14]. Moreover, when more refined criteria were used such as the rejection incidence during the antibody administration period [12] or the need for anti-rejection retreatment [9], as ALG had a nearly fully protective effect against rejection, but not the IL2 receptor-specific monoclonal antibody. The question is whether patients experiencing rejection under triple or quadruple therapy with P55-specific antibodies should be individualized as "immunologically high-risk" transplant recipients requiring heavy immunosuppressive protocols, or whether the relative lack of efficiency of IL2 receptor-specific monoclonal antibodies in some individuals is related to a pharmacokinetic interference such as an inadequate dose or the appearance of xenogeneic-specific antibodies inactivating the drug. Since the exact mechanisms by which IL2 receptor-specific monoclonal antibodies exert their action are still poorly understood, and probably vary between the different similarly available molecules, both explanations are advisable. If the lack of cytotoxicity of P55-specific antibodies can account for a relative lack of efficacy in some individuals, we can expect new strategies to improve antibody potency such the addition of a toxin molecule [8]. Another possibility is to attempt to reduce the immunogenicity of xenoantibodies. Recently, humanized anti-Tac has been injected into primates [5]. Results support the view that such chimeric monoclonal antibodies will avoid the immune response and improve its pharmacokinetic value. However, if our data deal with previous reports on the strong immunogenicity of IL2 receptor-specific rat [12] or murine [9] antibodies, it should be noticed that only four patients (27%) developed an IgG response by the end of the LO-Tact-1 administration. Moreover, the presence of LO-Tact-1-specific IgG did not correlate with low antibody trough levels nor with a further occurrence of allograft rejection and finally did not influence the graft outcome. On the other hand, we found that patients, who acutely rejected their graft had lower LO-Tact-1 levels and increased soluble IL2 receptor levels. Thus, close biological monitoring can provide helpful information during the administration of LO-Tact-1, since in a given patient a higher dose may be required to achieve efficient circulating and in situ concentrations.

In conclusion, the LO-Tact-1 anti-IL2 receptor monoclonal antibody administered in the prophylaxis of renal allograft rejection in combination with other conventional immunosuppressants was perfectly tolerated, did not induce severe infections related to overimmunosuppression and had a comparable effect in preventing rejection to the powerful quadruple combination CsA, AZA, high dose steroids, and polyclonal ALG. A randomised prospective study including a large number of patients is in progress in our centre, in order to confirm these preliminary results.

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