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A short-term combination therapy with cyclosporine and rapamycin or leflunomide induces long-term heart allograft survival in a strongly immunogenic strain combination in rats

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Abstract Synergism between cyclosporine (CsA) and rapamycin (RAPA) or leflunomide (LF) was studied in a strongly immunogenic cardiac allograft model in rats. In the absence of immunosuppression, PVG recipients rejected WKAH heart grafts after a mean survival time (MST) of 5.2 ± 1.1 days. A dose of 7.5 mg/kg per day CsA did not prolong graft survival (MST 5.6 ± 1.2 days). CsA given at 10 mg/kg per day for 30 days extended MST of the grafts to 48 ± 7 days. A short course of combination therapy consisting of adding a non-therapeutic dose of RAPA or a subtherapeutic dose of LF to a 1-month course of CsA resulted in permanent graft survival. These data suggest that RAPA and LF synergize with CsA enabling not only the lowering of the dose of CsA, but also inducing transplantation tolerance.

Key words Allograft · Tolerance · Cyclosporine · Rapamycin · Leflunomide

Introduction

Efficient T lymphocyte activation requires two signals. Signal 1 is provided by an interaction between the T cell receptor (TCR) and antigens in association with self-MHC molecules (TCR-antigen pathway). Signal 2 results from an interaction between non-MHC molecules and their ligands. As a costimulatory signal, signal 2 determines whether T lymphocytes undergo mitosis, apoptosis or anergy. It is well recognized that the CD28–B7 interaction is one of the most important in signal 2 transduction. Cyclosporine (CsA) and FK506 suppress T lymphocyte activation mainly by blocking the TCR-antigen pathway [1]. In contrast, rapamycin (RAPA) can also block the CD28–B7 pathway [2]. Leftunomide (LF), an isoxazol derivative which recently raised a lot of interest as a potential immunosuppressant, is also believed to be able to block the CD28–B7 pathway [3].

In various rat strain combinations, transplantation tolerance can be readily induced using CsA or RAPA monotherapy [4]. However, we found that in a strongly immunogenic strain combinations, induction of the tolerance using only one drug appeared to be more difficult. Hence, we investigated the potential of drugs blocking signal 2, such as RAPA and LF, to synergize with CsA which blocks signal 1 in this highly immunogenic model.

Materials and methods

T lymphocyte proliferation in vitro

The mixed lymphocyte reaction (MLR) as well as the proliferation assay of purified T cells through the CD3 and CD28 pathways were performed on human peripheral blood mononuclear cells (PBMC) using the methods described elsewhere [3].

Animals

Inbred male PVG rats ($RT1^c$), weighing 200–250 g were used as recipients. Inbred WKAH rats ($RT1^k$), weighing 100–150 g were used as donors.

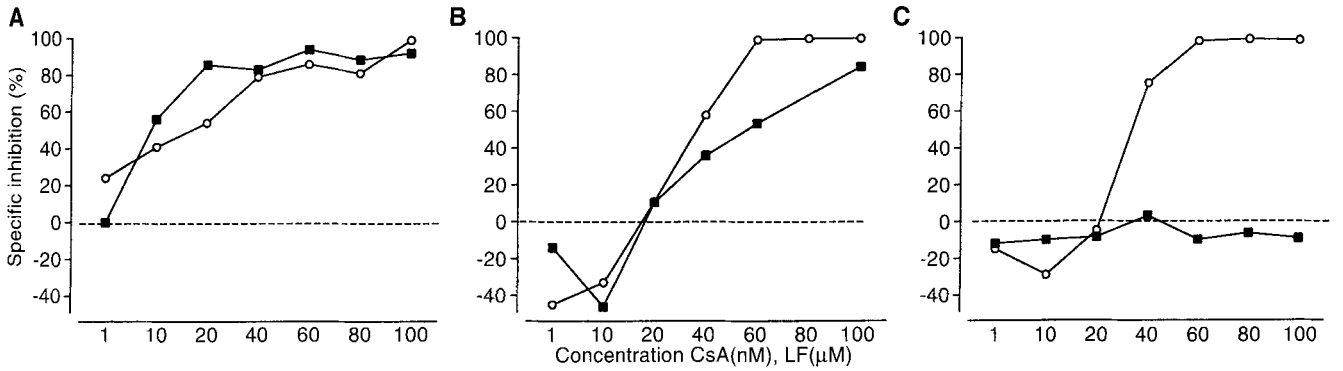

Fig. 1 A–C Effect of CsA and LF on human T cell proliferation in vitro. **A** T cell proliferation in MLR. **B** Purified T cell proliferation by stimulation with OKT3 + PMA. **C** Purified T cell proliferation by stimulation with anti-CD28 + PMA. (■ Inhibition mediated by CsA, ○ inhibition mediated by LF)

Heterotopic heart transplantation

Heterotopic heart transplantation was performed by implanting the heart grafts into the neck of recipients using methods described previously [5]. The function of grafts was monitored by daily inspection and palpation. Rejection was determined by the cessation of the graft beating and confirmed by histology.

Immunosuppressive therapy

LF (HWA 486) and RAPA were freshly suspended in 1% carboxymethylcellulose and given by gavage and i.p., respectively. CsA was diluted in olive oil and given by gavage.

Results

Effect of CsA and LF in different T cell stimulation pathways

CsA and LF both inhibited T cell proliferation in a dose-dependent manner in the MLR (Fig. 1 A, B), as well as through the CD3 (OKT3) stimulation pathway (Fig. 1 C). In contrast, the CD28 pathway was only inhibited by LF. These results are similar to those previously published for RAPA and confirmed what has been reported for LF (Fig. 1 C) [3].

Effect of CsA, RAPA and LF on graft survival

Graft survival is shown in Fig. 2. Untreated PVG rats rejected WKAH heart allografts after a mean survival time (MST) of 5.2 ± 1.1 days. CsA at 7.5 mg/kg per day and RAPA at 2.5 mg/kg per day did not affect graft survival (MST 5.6 ± 2 and 4.2 ± 1 days, respectively) (Fig. 2 A). CsA and LF both given at 10 mg/kg per day for 30 days prolonged graft survival to 48 ± 7 and 32 ± 7.4 days, respectively (Fig. 2 B). Combination of RAPA (2.5 mg/kg per day, day 0–15) with CsA (7.5 mg/kg per day, day 0–30) (Fig. 2 A) or combination of LF (10 mg/kg per day, day 0–30) with CsA (10 mg/kg per day, from day 0–30) (Fig. 2 B) resulted in indefinite graft survival.

Discussion

Looking for new immunosuppressants which can provide additive or synergistic effects on CsA remains an option for reducing the side effects of CsA and so achieving better immunosuppression. As T cell activation is now generally believed to be dependent on two activation pathways and as CsA and FK506 only suppress the first one (delivered by the TCR), much interest exists in drugs that can suppress the second pathway (delivered by the B7–CD28 interaction). RAPA and LF clearly seem to belong to the latter class of the drugs as was shown previously [2, 3], and this is also confirmed in the present study.

Here we show that in a strongly immunogenic heart allograft model in rats, synergism between the two types of drugs is also observed in vivo. The immune mechanisms involved are not precisely known as yet, but they seem to be different from what was previously found in a weaker immunogenic rat combination also using the RAPA + CsA combination therapy [6]. Indeed, in contrast to the latter study, we found no arguments for the presence of suppressor cells, nor for the existence of circulating blocking antibodies. It, therefore, seems that a combination therapy of immunosuppressants blocking signal 1 and signal 2 of T cell activation, respectively, may lead to long-term graft survival without maintenance therapy based on different immune mechanisms. The mechanism responsible for the long-term graft survival in our study is at present being further investigated.

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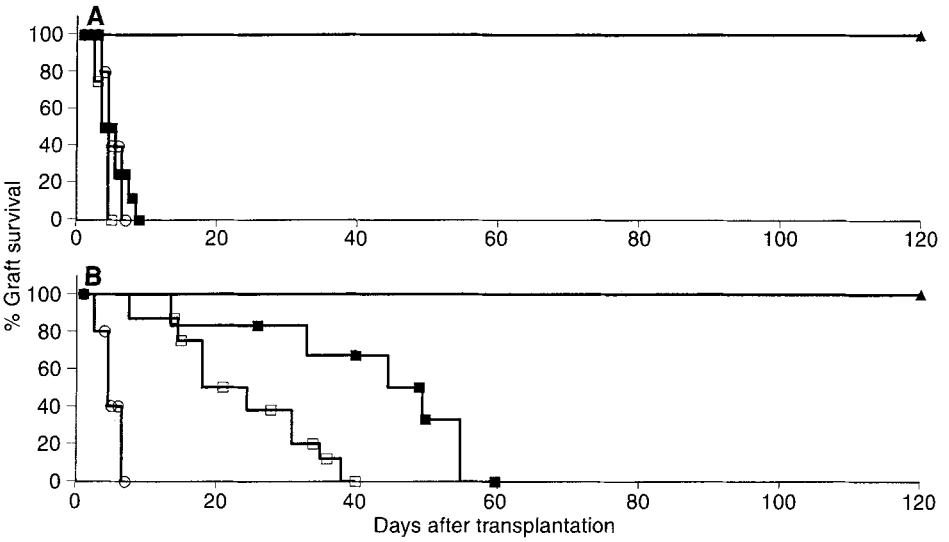

Fig. 2 **A** Effect of CsA and RAPA given alone or in combination on graft survival; no treatment (○) CsA 7.5 mg/kg per day (■), RAPA 2.5 mg/kg per day (□), combination of CsA (day 0–30) and RAPA (day 0–15) (▲). **B** Effect of CsA and LF given alone or in combination on graft survival; no treatment (○), CsA 10 mg/kg per day, day 0–30 (■), LF 10 mg/kg per day, day 0–30 (□), combination of CsA (day 0–30) and LF (day 0–30) (▲)

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