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ORIGINAL ARTICLE

Association of interleukin4 gene polymorphisms of recipients and donors with acute rejection following living donor liver transplantation

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Summary
Background: Little is known as to whether the interleukin4 (*IL4*) gene polymorphisms in recipients or donors affect the incidence of acute cellular rejection (ACR) following living donor liver transplantation (LDLT). Therefore, we determined the effect of *IL4* T-33C polymorphisms in recipients and donors on ACR in a large cohort of patients that underwent LDLT.
Methods: We examined 155 LDLT cases treated at Nagoya University or Kyoto University, Japan, between 2004 and 2009. *IL4* T-33C polymorphisms were analyzed in recipients and donors.
Results: Forty-seven recipients (30.3%) developed early ACR. The genotype frequency of *IL4* T-33C in the recipients was associated with ACR incidence ($P = 0.008$, $P < 0.0125$ considered significant). Patients with the *IL4*-33C carrier genotype (C/C or C/T) were significantly associated with a higher incidence of ACR relative to those with the T/T genotype (OR = 3.27, 95% CI: 1.56—6.88, $P = 0.002$). The genotype frequencies of *IL4* T-33C in the donors were not associated with rejection incidence. In addition, there was no significant effect of *IL4* T-33C genotype combinations on ACR incidence in donors and recipients.
Conclusions: Genotyping of *IL4* T-33C in recipients might be useful to stratify the liver transplant recipients according to their risk of ACR.

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Introduction

Cytokine genotypes have been previously studied in patients who have undergone solid organ transplantation, and certain polymorphisms have been found to affect the development in graft rejection [1—10]. In the field of organ transplantation, pro-inflammatory Th1 cytokines are believed to be involved in acute transplant rejection, by directly contributing to graft damage. In contrast, the Th2 subset of cytokines mediates induction of tolerance toward the transplanted organ by suppressing Th1 responses [8,11,12].

Interleukin (IL)-4, which is a prototypic member of the Th2 cytokines, is a potent anti-inflammatory and is an anti-inflammatory cytokine that exerts immunosuppressive effects on macrophages and suppresses pro-inflammatory cytokine production [13,14]. Several polymorphisms have been found in the *IL4* gene, and the promoter polymorphism *IL4* T-33C, which is a complete linkage disequilibrium with *IL4* T-589C, affects IL-4 expression [13,15].

Several studies have investigated the risk of recipient *IL4* gene polymorphisms in acute rejection of allografts [1,8—10,16,17]. In addition, studies have assessed the association of donor *IL4* polymorphisms and acute rejection of solid organ allografts [9,10,17,18]. Poole et al. demonstrated a significant decrease in the donor *IL4*T positive genotype (T/T + C/T) of T-590C in the rejecter group of renal transplantation [17]. Bijlsma et al. found that patients who received a heart transplantation from donors with the −590T-positive genotype underwent significantly less from rejection [18]. However, in terms of liver transplantation (LT), few studies have investigated the relationship of *IL4* gene polymorphisms in recipients and donors with the incidence of acute cellular rejection (ACR).

Despite recent improvements in anti-rejection medications, ACR remains a major complication associated with morbidity and late graft loss in patients who undergo LT [19,20]. Furthermore, despite adequate treatment, approximately 5—10% of liver transplant recipients who develop ACR progress to severe ductopenic rejection [21].

However, immunosuppressive medication can induce significantly adverse side effects. Although calcineurin inhibitors (CNIs) are currently the mainstay for immunosuppression in liver transplant recipients, CNI-induced renal failure is a serious problem following transplantation, and low doses of immunosuppression are suggested along with careful monitoring [22]. Alternative approaches to diminish CNI exposure and improve renal function include delayed CNI dosing or withdrawal and substitution with an alternative agent such as mycophenolate mofetil (MMF) or Sirolimus [23]. However, these strategies may be associated with an increased risk of acute rejection. Thus, it is important to balance the risk of ACR with the risk of drug toxicity. Safe reduction of immunosuppressive drugs tailored to specific patient needs is crucial to improve outcomes, whereas identification of risk factors may permit a more individualized approach to immunosuppressive therapy.

In the present study, we determined the effects of *IL4* T-33C polymorphisms in recipients and donors on ACR in a large cohort of recipients who underwent living donor liver transplantation (LDLT).

Materials and methods

Patients

We investigated recipients and donors who underwent LDLT at Nagoya University or Kyoto University between 2004 and 2009, and cases in which the recipient survived for at least 6 months after transplantation. We excluded ABO-incompatible cases as well as patients who received a steroid-free immunosuppressive regimen for another study. The procedures for recipients and living donors in LDLT have been described elsewhere [24,25]. Immunosuppressive drugs including tacrolimus or cyclosporine, and steroids were administered after transplantation. The trough level of tacrolimus was targeted at 10—15 ng/mL for the first 2 weeks and then 5—10 ng/mL for the following 2 months. Cyclosporine was administered intravenously for the first 2 weeks and then orally with C0 and C2 level monitoring. The target C2 level of cyclosporine was set between 500 and 800 ng/mL for the first month. When the C0 level of cyclosporine exceeded 300 ng/mL, the cyclosporine dosage was appropriately reduced.

The initial administration of methylprednisolone was intravenously given at a dose of 10 mg/kg immediately after reperfusion, followed by 1 mg/kg twice a day for the first 3 days, and tapered within the first week. Thereafter, oral prednisone was administered at a dose of 0.3 mg/kg once a day and tapered. All procedures were in accordance with the ethical standards of the responsible committee on human experimentation and the Helsinki Declaration of 1975 as revised in 2008 [26]. This study was approved by the ethics committees of Nagoya University School of Medicine (approval number: 290) and Kyoto University (approval number: G177). Signed informed consent was obtained from each recipient and donor who participated in this study.

Acute cellular rejection

We analyzed the occurrence of ACR within 6 weeks after LT. A liver biopsy was obtained from patients with worsening results in liver function tests and/or symptoms suggestive of ACR without another cause. ACR diagnosis was confirmed by liver biopsy findings according to the Banff schema [27]. Upon confirmation of ACR, treatment was generally initiates with high-dose corticosteroids, followed by elevation of the daily immunosuppressive medications by increasing the dose of CNI or starting MMF. Muromonab (OKT3) was usually administered to patients with steroid-resistant rejection.

Genotyping

Genomic DNA was extracted from peripheral blood samples obtained from the recipients, whereas genomic DNA samples of the donors were isolated from peripheral blood samples or liver biopsy specimens of the graft obtained during donor surgery. DNA was extracted from a heparinized buffy coat with a BioRobot M48 Workstation (Qiagen, Tokyo). Isolated DNA was used for genotyping *IL4* T-33C polymorphisms by polymerase chain reaction with confronting two-pair primers (PCR-CTPP) as described previously [13,28—30].