

Tacrolimus May Induce the Production of Nucleolar Anti-nuclear Antibody in Liver Transplant Patients

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Abstract

Background & Aims: Immunosuppressive drugs have been used to prevent graft rejection in most allo-liver recipients and the immune state of these patients differs greatly before and after transplant operation. This study aims at evaluating the immune state of liver transplant patients treated with tacrolimus by investigating the production of anti-nuclear antibodies (ANA). **Methods:** A hundred and eighty-eight serum samples from 94 allo-liver recipients treated with tacrolimus and from 94 patients with matched liver diseases were tested for ANA by indirect immunofluorescence assay with HEp-2 cells as substrate. **Results:** ANA were detected as positive in 20.2% of the liver transplant patients treated with tacrolimus, and in 12.8% of disease-matched control patients, but this difference was not statistically significant ($P=0.17$). However, the frequency of nucleolar ANA pattern in ANA-positive cases was significantly higher in the liver transplant patients (63.2%) than in the control group (16.7%) ($P=0.01$). **Conclusion:** Tacrolimus may contribute to producing nucleolar ANA in liver transplant patients. The autoimmune disease susceptibility of allo-liver recipients treated with tacrolimus requires further studying.

Key words

Tacrolimus – liver transplant – nucleolar ANA.

Introduction

About 8% of the Chinese population, nearly 100 million patients, suffer from hepatitis B or are carriers of the hepatitis B virus (HBV) [1]. A definite association of HBV infection

and liver cirrhosis or liver cancer has been demonstrated [2]. Both liver cirrhosis and cancer could cause liver dysfunction that severely impairs the quality of life of patients [3]. Liver transplantation nowadays is a widely accepted treatment option for liver cirrhosis and cancer [4]. After liver transplantation, immunosuppressive treatment is necessary to reduce the risk of subsequent graft rejection and to guarantee a long-term survival of exogenous liver in recipients.

Although the immunosuppressive regimens for all solid organ transplants are fairly similar, tacrolimus (also known as FK506) is one of the most popular immunosuppressants [5]. As a potent suppressant of T lymphocytes, tacrolimus is believed to regulate the immune state and balance of recipients after an operation. So the diseases related to the immune disorder, such as tumor and infection resulting from lower immunity, have drawn the attention of clinicians when applying a tacrolimus-based regime.

Up till now, the relationship between the tacrolimus treatment and development of autoantibodies or autoimmune diseases has not yet been deciphered. Since the 90s, several groups reported the presence of de novo autoimmune liver diseases as well as serum autoantibodies after liver transplantation and administration of immunosuppressive regimen [6-7]. However, the inducing effect of tacrolimus on autoimmune disease occurrence after liver transplantation remains to be clarified. Anti-nuclear antibodies (ANAs) constitute a group of important autoantibodies acting against the nucleus or cytoplasmic substances of nucleated cells. The production of these autoantibodies mostly occurs in a number of autoimmune disorders including systemic lupus erythematosus and Sjögren's syndrome [8-9]. The aim of the current study is to evaluate the immune state of allo-liver recipients by measuring ANA in the serum in two groups of liver transplant patients with a long-term tacrolimus treatment and disease-matched controls.

Patients and Methods

This study has been approved by the Ethics Committee of the West China Hospital of Sichuan University. Written informed consent was obtained from each participant.

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The hundred and eighty-eight liver transplant patients and patients in a control group were recruited from in-patients and out-patients of West China Hospital from 2007 to 2010. Patients from the control group were matched by means of age, gender, etiology, and disease. In the liver transplant group, 91 (96.8%) cases were detected to be HBV-positive with preoperative testing, while the control group counted 92 (97.9%) HBV-infected patients. All allo-recipients were treated with tacrolimus alone as an immunosuppressive agent. The patients with autoimmune liver disease such as autoimmune hepatitis (AIH), primary biliary cirrhosis (PBC) and patients with hepatitis C were excluded from the study due to a definitely high ANA prevalence.

The blood samples were collected in 4 ml BD Vacutainer without anticoagulant and allowed to clot up to 1 hour at room temperature. Within 2 hours after collection the samples were centrifuged at 1500g for 10 min at 4°C to collect serum. The sera were stored at -80°C and thawed at 4°C overnight prior to analysis.

ANA detection

ANA were determined by indirect immunofluorescence assay with commercialized ANA kit (EUROIMMUN Co., Germany) with HEP-2 cell lines as substrate at an initial serum dilution of 1:100 according to the manufacturer's instructions. The serum was further diluted as 1:320, 1:1000, 1:3200 and 1:10000 subsequently, when a positive result was obtained with the lower dilution. ANA fluorescence patterns were defined as speckled, homogeneous, nucleolar (Fig. 1), centromere, nuclear dots and cytoplasm staining, etc. ANA fluorescence pattern of each sample was observed in double blind with a fluorescence microscope (Model E6000 Nikon Co., Japan).

Statistical analysis

Quantitative data obeying a normal distribution were described by their mean and standard deviation (SD). When the distribution of data was skew, the median and range were used instead. The Mann-Whitney U or Chi-square test was applied for comparing both patient groups. A probability value (P) inferior to 0.05 was considered statistically significant. All data were analyzed with SPSS 16.0.

Results

Age, gender, and disease

The characteristics of the subjects enrolled in the trial are shown in Table I. There was no significant difference between liver transplant and control patients in age, gender, etiology, and disease distribution ($P > 0.05$). This indicates that the matching based on these 4 variables was adequate.

ANA detection

The incidence of ANA positivity was 20.2% and 12.8% respectively, in the groups of liver transplant patients treated with tacrolimus and the control patients. There was no statistical difference between these two groups ($P = 0.17$). However, the frequency of nucleolar pattern, which reached up to 63.2% in the ANA-positive liver transplant patients, was only 16.7% in the control group, indicating a significant

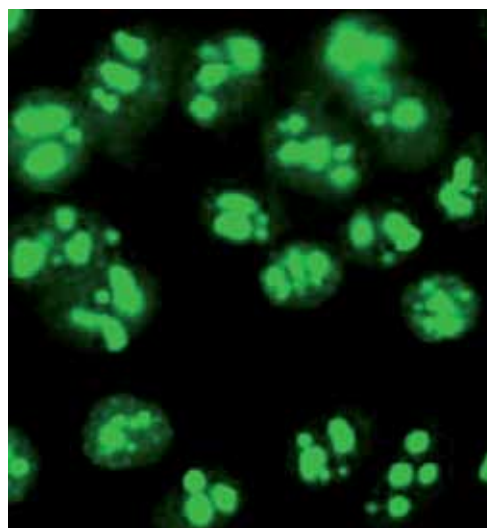


Fig 1. Example of nucleolar pattern of ANA detected by indirect immunofluorescence assay with HEP-2 cells as substrate (40×amplified).

difference between both groups ($P = 0.01$) (Table II). For all enrolled transplant patients, the median duration of tacrolimus treatment was 42 months, and the mean plasma concentration of the drug was 6.2 ± 2.4 ng/ml.

The transplant patients were divided into a positive subgroup with 19 cases and a negative subgroup with 75 patients based on the presence of ANAs. The median durations of treatment with tacrolimus were 36 and 42 months, respectively in the positive and negative subgroups. No significant difference was found between both subgroups ($P = 0.58$). The mean plasma concentrations of tacrolimus in the positive and negative subgroups were 5.8 ± 2.4 and 6.3 ± 2.4 ng/ml, respectively. The difference in mean (0.5 ng/ml) between both subgroups was not statistically significant ($P = 0.40$).

Discussion

Because of the risk of rejection after liver transplantation, immunosuppressive drugs are necessary for sustaining the graft function and improving the life quality of recipients. Tacrolimus, an effective calcineurin inhibitor, was approved for clinical use based on the merit of its immunosuppressive activity [10]. Its action mechanism can be readily explained. Tacrolimus binds to 12 kDa FK506-binding immunophilin protein (FKBP12) of type-3 ryanodine receptor resulting in a FK506-FKBP12 complex. This complex associates with the calcineurin with high affinity and inhibits its serine/threonine phosphatase activity, which results in the inhibition of the transcription of interleukin 2 and the blockade of Ca^{2+} dependent T lymphocyte activation pathway. The immunosuppressive activity of tacrolimus determines an impairment of T lymphocyte activation and proliferation [11], so that cellular and humoral immunity of liver transplant patients will be imbalanced after long-term usage of the drug.

Table I. Characteristics of the subjects and disease distribution

| Group | Number of cases | Male/female | Median age (range) | Etiology of HBV(%) | Disease distribution (%) | | |
|---------------------------|-----------------|-------------|--------------------|--------------------|--------------------------|--------------|-----------------------------------|
| | | | | | Cirrhosis | Liver cancer | Other liver diseases ^a |
| Liver transplant patients | 94 | 79/15 | 44 (24 ~ 70) | 91 (96.8%) | 52.1% | 14.9% | 33.0% |
| Control | 94 | 79/15 | 46 (24 ~ 78) | 92 (97.9%) | 55.3% | 14.9% | 29.8% |

Note: ^a including severe hepatitis, liver failure, intrahepatic bile duct stone and severe liver rupture etc. excluding autoimmune liver disease, AIH, PBC and Hepatitis C.

Table II. ANA prevalence and titer in liver transplant patients and controls

| Group | Number of cases | ANA prevalence (%) | Median titer of ANA [range] | Nucleolar pattern prevalence of ANA positive (%) ^a | Median titer of nucleolar pattern [range] | Other pattern prevalence of ANA positive ^b |
|---------------------------|-----------------|--------------------|-----------------------------|---|---|---|
| Liver transplant patients | 94 | 19 (20.2%) * | 100 (100~1000) ** | 12/19 (63.2%) † | 100 (100~320) †† | 7/19 (36.8%) |
| Control | 94 | 12 (12.8%) * | 100 (100~320) ** | 2/12 (16.7%) † | 210 (100~320) †† | 10/12 (83.3%) |

Note: ^a including pure nucleolar pattern and nucleolar pattern mixed with others; ^b Other pattern including speckled, centromere, cytoplasm and homogeneous pattern etc; * $\chi^2 = 1.89$, $P = 0.17$; ** $Z = -0.26$, $P = 0.80$; † $\chi^2 = 6.42$, $P = 0.01$; †† $Z = -0.21$, $P = 0.83$.

Furthermore the transplanted liver as an exogenous antigen could stimulate and activate the patients' immune responses [12]. Consequently, the patients' own immune balance would be re-regulated because of the foreign organ and tacrolimus usage. In order to achieve a new immune equilibrium, the immune system including immune molecules, cells, and organs would be extensively regulated as compared with that before transplantation [13-15]. Since the immunological tests, such as the ANA test, were not performed for most patients before transplantation, it was impossible to design a self-controlled study. Therefore, it was decided to choose patients with matched liver diseases as controls in this trial.

Antinuclear antibodies are one of the effective indicators to evaluate the disorders of immune system resulting from the imbalance of immunity against the own cellular antigens [16]. Normally, a positive result of ANA test may suggest the emergence of auto-reactivity due to primary autoimmune or drug-induced autoimmune disorders [17]. Because of the high prevalence of ANA in autoimmune liver diseases and hepatitis C, patients with these disorders were excluded from both groups of liver transplant and control patients in order to diminish the non-specific influence of these diseases on the ANA incidence studied in the current trial [18-19]. The overall incidences and median titers of ANA in the liver transplant group were comparable to those of the control group. However, it could not be concluded that the immune state of the liver transplant patients was similar to that of the control patients.

Antinuclear antibodies represent a spectrum of autoantibodies directed against various cellular components [20]. Different ANA patterns were determined according to typical fluorescence staining patterns [21]. The frequency of the nucleolar pattern was significantly higher in liver transplant patients compared with the control patients in this study, although nucleolar pattern is reported as a rare pattern of ANA, especially in autoimmune diseases [22]. Despite the

well known association between nucleolar ANA pattern and scleroderma [23, 24], no liver transplant patients treated with tacrolimus had obvious scleroderma-related symptoms.

Since anti-smooth muscle antibody (SMA) and liver kidney microsomal antibody (LKM) have also been detected besides ANAs in some post-liver transplantation cases with immunosuppressive regimens [6-7], a further study concerning these autoantibodies and the related autoimmune diseases in allo-liver recipients treated with tacrolimus would be necessary to clarify the autoimmunity-inducing effect of tacrolimus.

Conclusions

The current study demonstrates that the long-term administration of tacrolimus in liver transplant patients induces the production of nucleolar ANA. As a suggestion, a set of autoantibodies, including ANA, should be regularly monitored in liver transplant patients treated with tacrolimus for monitoring the impact of this drug on disease prognosis and, if necessary, in time decreasing the dose.

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Conflicts of interest

None to declare.

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