Transpl Int (1998) 11 [Suppl 1]: S185–S188

M. Hirata
Y. Kita
S. Saito
M. Nishimura
M. Ito
K. Mizuta
H. Tanaka
Y. Harihara
H. Kawarasaki
K. Hashizume
M. Makuuchi

Increase in natural killer cell activity following living-related liver transplantation

M. Hirata (✉) · Y. Kita · Y. Harihara · M. Makuuchi
Second Department of Surgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan
Fax +81-3-5684-3989

S. Saito · M. Nishimura
Department of Research, Japanese Red Cross Central Blood Center, 4-1-31 Hiroo, Shibuya-ku, Tokyo 150, Japan

M. Ito · K. Mizuta · H. Tanaka · H. Kawarasaki · K. Hashizume
Department of Pediatric Surgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan

Abstract We monitored the serial changes of natural killer cell (NK) activity in eight recipients of living-related liver transplantation. The HLA types of all eight patients were haplotypically identical with those of their donors. Tacrolimus and methylprednisolone were used for immunosuppression. The NK activity before transplantation was 24.1 ± 20.2% which is surprisingly low when compared with the value for normal individuals (67.7 ± 13.2%, $P < 0.01$) or a liver dysfunction group (49.4 ± 21.9%, $P < 0.05$). Serial changes in NK activity revealed a minimum of 6.1 ± 3.6% 1 week after transplantation, gradually increasing to 49.2 ± 12.5% at 2 months after transplantation. These results suggest that the diseased liver might play an important role in the suppression of NK activity.

Key words NK activity · Living-related liver transplantation

Introduction

Natural killer cell (NK cell) activity is believed to play an important role in organ transplantation and host anti-cancer defense mechanisms. After renal transplantation, NK cell activity decreases if no rejection episodes occur, and this lowered NK activity may have some relationship with the high frequency of cancer in renal transplant recipients [4]. If rejection does occur, NK cells are activated and NK activity increases [12, 13]. However, it remains difficult to interpret changes in NK activity because many factors, such as alloreactivity, immunosuppressive drugs, surgical stress, and infection, may influence the immune reaction.

In contrast, few reports have been published about alterations in NK activity before and after liver transplantation [14]. In addition, NK activity in patients with liver cirrhosis or end-stage liver disease is lower than that in normal individuals [3, 5, 6]. In order to determine whether liver disease really does affect NK activity, we monitored serial changes in NK activity in patients who underwent living-related liver transplantation.

Patients and methods

The living-related liver transplant recipients consisted of eight patients suffering from end-stage cholestatic liver diseases (six men and two women, mean age 9.1 ± 9.0 years; Table 1). The HLA types of all eight patients were haplotypically identical with those of their donors. ABO types were also identical except in patient 4. The lymphocyte cross-match test was negative in all cases. No patient was taking any immunosuppressive drugs before transplantation and none exhibited any symptoms of infection for at least 2 weeks before transplantation.

Living-related liver transplantation was performed as previously reported [7]. The whole liver was removed from each recipient and the left lobe (patients 1, 2, 8) or the left lateral lobe (patients 3–7) of the donor's liver was transplanted to the recipient.

Tacrolimus (FK506) and methylprednisolone were used for immunosuppression. The dose of FK506 used after transplantation

Table 1 Characteristics of living-related liver transplant recipients (*NK* natural killer cell, *PBC* primary biliary cirrhosis, *BA* biliary atresia)

Case	Age	Gender	Disease	Donor	Serum TB	Serumbile acids (μmol/l)	NK activity (%)
1	25 years	Male	PBC	Mother	11.8	144	10.9
2	18 years	Female	BA	Mother	3	124	4.8
3	4 years	Female	Alagille	Grandmother	12.5	668	36.1
4	10 months	Male	BA	Father	16.4	159	10.7
5	10 months	Male	BA	Mother	14.9	179	8.8
6	10 months	Male	BA	Father	20.1	122	15.8
7	8 years	Male	BA	Mother	17.9	104	56.4
8	14 years	Male	BA	Mother	8.3	199	49.3

was based on the whole-blood concentration of the drug. The blood level of FK506 was controlled at 15–20 ng/ml [0–7 postoperative days (POD)], 12–18 ng/ml (8–14 POD), 10–15 ng/ml (15–28 POD), and 5–10 ng/ml (after 28 POD). The methylprednisolone dose was 40 mg/kg on the day of operation, and 3.0 mg/kg on POD 1, gradually tapering to 0.25 mg/kg per day by 1 month posttransplantation.

As a normal control for NK activity and the liver dysfunction group, mononuclear lymphocytes were taken from nine normal volunteers (eight men and one woman, mean age 40.2 ± 8.4 years) and 14 patients with chronic liver disease (12 men and 2 women, mean age 54.3 ± 13.8 years, eight with liver cirrhosis and six with chronic hepatitis).

The study was performed in accordance with the ethical standards set down in the 1964 Declaration of Helsinki. All participants gave their informed consent prior to their inclusion in the study.

Measurement of NK activity

Samples of peripheral venous blood were obtained in ACD collection tubes from all subjects at the same time each morning (8:00 a.m.) 1 week before transplantation, and 1 week, 3 weeks, and 2 months after transplantation. Peripheral blood mononuclear cells were isolated from these samples using Ficoll-Hypaque gradients (Ficoll-Paque; Pharmacia Biotek, Sweden; specific gravity 1.3545). NK activity was measured immediately after isolation. The concentrations of these cells were adjusted for testing in RPMI1640 containing 10% fetal calf serum.

The target cells used in the assay were K562. K562 cells were maintained in continuous culture in complete medium containing 10% fetal calf serum. Immediately before staining, dead cells were removed by spinning on Ficoll-Hypaque gradients for 10 min at 3000 rpm.

For the microcytotoxicity assay, the target cells were stained with carboxyfluorescein diacetate, according to the modified technique of Brunning et al. [1]. The microcytotoxicity assay was performed on the same day as the samples were obtained. Effector cells were adjusted to 8.0×10^6 cells/ml in 100-μl volumes, and added in triplicate to wells in 96-well trays (Nunclon; Inter Med, Denmark; 8.0×10^5 cells/well). Stained target cells were adjusted to 1.0×10^5 cells/ml in 100-μl volumes and added to the wells (1.0×10^4 cells/well). The effector to target (E:T) ratio was thus 80:1. Assays at E:T ratios of 40:1, 20:1, and 10:1 were also performed at the same time. Wells to which no effector cells had been added were used as negative controls, while wells to which Triton X-100 had been added were used as positive controls. After spinning for 5 min at 800 rpm, the trays were incubated for 1 h in a 37 °C, 5% CO_2 incubator. At the end of the reaction, the trays were centrifuged at 1500 rpm for 5 min to pack the cell layer, then 100 ml of media was carefully taken from each well and added to an empty well of a new 96-well tray (Cell Wells; Corning Glass Works, Corning, New York, USA). The trays were immediately analyzed in an automatic microfluorometer (Titertek Fluoroscan II, Labsystems, Helsinki, Finland). Cytotoxicity was expressed as the percentage label release, calculated from the following formula: 1–[(mean experimental – mean negative control) / (mean positive control – mean negative control)] × 100.

Measurement of phenotypes effector cells

At the same time as measurement of NK activity, two-color flow cytometry was performed on effector cells. To 1.0×10^6 of washed in phosphate-buffered saline (PBS), cells 10 μl of fluorescein isothiocyanate or PE-labeled antibodies (anti-CD3, CD16, CD56; Immunotech, Westbrook, Me., USA) was added and incubated for 30 min at room temperature. Cells were washed and resuspended in 200 μl of PBS. These samples were immediately analyzed on a FACSCAN flow cytometer (Becton Dickinson, San Jose, Calif., USA).

Statistics

The Wilcoxon test for unpaired data was used for statistical analysis and the level of significance was set at $P < 0.05$.

Results

No histologically proven episodes of rejection were observed. Patient 1 cytomegalovirus developed (CMV) infection on POD 41. He was treated with ganciclovir and CMV hyperimmune globulin. The seven remaining patients exhibited no symptoms of infection.

The NK activity in liver transplant recipients before transplantation (E:T = 80:1) was 24.1 ± 20.2% (Fig. 1). This value is surprisingly low when compared with the value for normal individuals (67.7 ± 13.2%, $P < 0.01$) or the liver dysfunction group (49.2 ± 12.5%, $P < 0.05$). Serial changes in NK activity in the transplant recipients (Fig. 2) revealed a minimum of 6.1 ± 3.6% 1 week after transplantation, gradually increasing to 49.2 ± 12.5% at 2 months after transplantation. The same trends were found at other E:T ratios (data not shown).

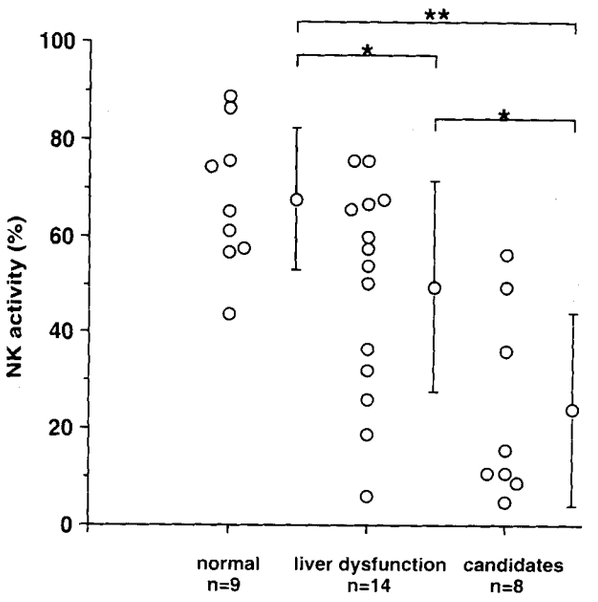


Fig. 1 Comparison of natural killer cell (NK) activity between normal individuals, the liver dysfunction group, and candidates for living-related liver transplantation [effector to target ratio (E:T) = 80:1, * $P < 0.05$, ** $P < 0.01$]

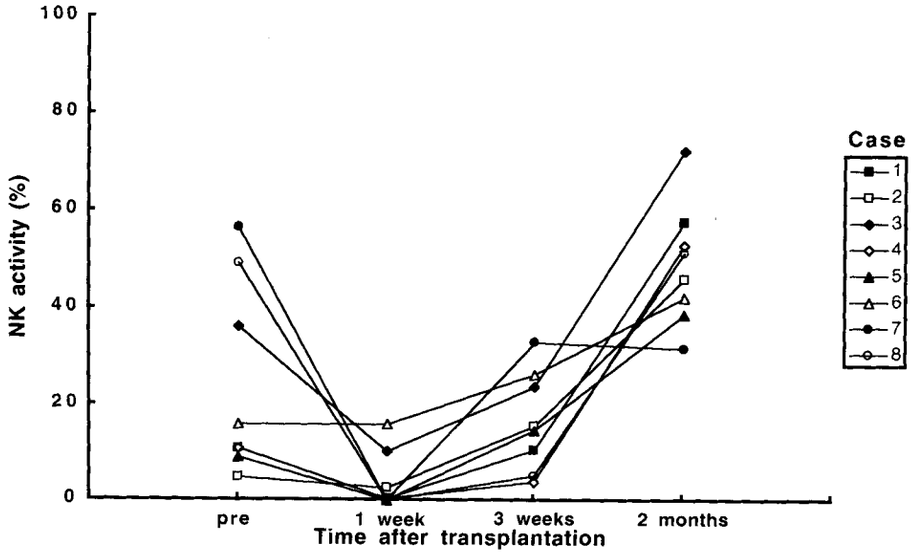
Before transplantation, the percentages of CD3-positive cells and CD3-negative,CD56-positive cells of transplant recipients were 45.5 ± 21.5 % and 12.4 ± 5.7 %, respectively. These values were comparable to those of normal individuals and there were no significant changes of phenotypes of peripheral blood mononuclear lymphocytes after transplantation (data not shown).

Discussion

In renal transplant recipients, several investigators have reported that NK activity before transplantation is comparable to that in normal individuals, but decreases after transplantation, and this lowered NK activity continues for more than 2 years [4, 12, 13]. NK activity recovered in the long term in some patients [4]. Other investigators have reported that lymphocytes taken from long-term renal transplantation patients have suppressor activity against the NK activity of normal individuals [9]. In cadaver liver transplantation, Whiteside et al. [14] reported that NK activity decreased from immediately after transplantation to at least 180 days after transplantation.

In contrast to the situation after renal or cadaver liver transplantation, our results showed that pretransplantation NK activity was surprisingly low in living-related liver transplantation candidates, while after transplantation it increased in spite of the use of FK506 and methylprednisolone (Fig. 2). Patients with end-stage liver diseases exhibit lower NK activity than normal individuals [3, 5, 6]. As shown in Fig. 1, the NK activity in patients with liver dysfunction was significantly lower than that in normal individuals. Patients with cholestatic liver disease congestion have also been considered to be in an immunosuppressed state [2], and some investigators have shown that bile acids suppress NK activity [10]. All eight recipients in our study had high blood concentrations of bile acid (212.4 ± 186.8 μmol/l, normal range 0–11). After transplantation, the blood bile acid concentration rapidly decreased almost to the normal range (15.2 ± 9.1 μmol/l). This might explain why the NK activity in all recipients increased at 2 months after transplantation, although all patients with high bile

Fig. 2 Serial changes in NK activity in living-related liver transplant recipients (E:T = 80:1)



acid concentration do not always reveal the lowest level of NK activity.

Surgical stress can also have a suppressive effect on NK activity. In non-immunosuppressed patients, NK activity lowered in response to surgical stress almost recovered within 7 days [4]. Surgical stress is severe in transplant patients, so this could well be one of the causes of the decrease in NK activity at 1 week after transplantation. However, surgical stress did not appear to have much influence on NK activity more than 1 week after transplantation.

At 1 week after transplantation, high doses of FK506 and methylprednisolone were used and NK activity reached its lowest level. However, at 2 months after transplantation, the blood concentration of FK506 was maintained at 5–10 ng/ml and the dose of methylprednisolone was 0.25 mg/kg per day. Even at such a low dose, FK506 still has some immunosuppressive effect on NK [11] and cytotoxic T lymphocyte activity [8]. This might explain why NK activity at 2 months did not reach the level of normal individuals.

In contrast to cadaver transplantation, in living-related transplantation, the HLA types of the recipients are haplotypically identical with those of the donors. None of the eight recipients developed any symptoms of rejection. This might explain the difference in serial NK activity changes between living-related and cadaver liver transplant recipients.

NK activity changes are very important when rejection occurs. In renal transplantation, some investigators have reported that NK activity increases during rejection, while others have found no relationship between NK activity and rejection. Further studies are needed to clarify the roles of NK activity in organ transplantation.

In conclusion, NK activity was suppressed in patients with end-stage cholestatic liver diseases, while it increased after living-related liver transplantation. These results suggest that diseased liver might play an important role in the suppression of NK activity.

Acknowledgements Supported in part by grants-in-aid for scientific research from the Ministry of Education, Science and Culture, and from the Ministry of Health and Welfare of Japan (08407036, 08457294, 07671292)

References

1. Brunning JW, Kardol MJ, Arentzen R (1980) Carboxyfluorescein fluorochromasia assays. I. Non-radioactively labeled cell-mediated lympholysis. J Immunol Methods 33: 33–44
2. Calmus Y, Weill B, Ozier Y, Chereau C, Houssin D, Poupon R (1992) Immunosuppressive properties of chenodeoxycholic and ursodeoxycholic acids in the mouse. Gastroenterology 103: 617–621
3. Chuang W, Liu H, Chang W, Chen S, Hsieh M, Wang L (1991) Natural killer activity in patients with liver cirrhosis relative to severity of liver damage. Dig Dis Sci 36: 299–302
4. Guillou PJ, Hegarty J, Ransden C, Davison AM, Will EJ, Giles GR (1982) Changes in human natural killer activity early and late after renal transplantation using conventional immunosuppression. Transplantation 33: 414–421
5. Hata K, Thiel DH van, Herberman RB, Whiteside TL (1991) Natural killer activity of human liver-derived lymphocytes in various liver diseases. Hepatology 14: 495–503
6. Hirofuji H, Kakumu S, Fuji A, Ohtani Y, Murase K, Tahara H (1987) Natural killer and activated killer activities in chronic liver disease and hepatocellular carcinoma: evidence for a decreased lymphokine-induced activity of effector cells. Clin Exp Immunol 68: 348–356
7. Makuuchi M, Kawarazaki H, Iwanaka T, Kamada N, Takayama T, Kumon M (1992) Living related liver transplantation. Surg Today 22: 297–300
8. Nakata S, Shirakura R, Ito T, Fukuzawa M, Miyagawa S, Yamamoto S, Nozaki T, Matsumiya G, Izutani H, Matsuda H (1994) Immunosuppressive mechanisms of deoxymethylspoergualin and FK506 on in vitro cytotoxic T lymphocytes. Transplant Proc 26: 1930–1932
9. Ono Y, Kerman RH, Kahan BD (1982) Natural cell-mediated cytotoxicity and suppressor activity for natural cell-mediated cytotoxicity in long-term renal transplant patients. Transplantation 34: 103–107
10. Podevin P, Calmus Y, Bonnefis MT, Veyrunes C, Chereau C, Poupon R (1995) Effect of cholestasis and bile acids on interferon-induced 2',5'-adenylate synthetase and NK activities. Gastroenterology 108: 1192–1198
11. Takaori K, Inoue K, Nio Y, Aung T, Fukumoto M, Hashida T, Yasuhara M, Hori R, Tobe T (1992) Basic study on immunologic effects of cyclosporine and FK506 for application to pancreatic transplantation. Transplant Proc 24: 894–896
12. Waltzer WC, Bachvaroff RJ, Anaise D, Rapaport FT (1984) Natural killer activity after renal transplantation. Transplant Proc 16: 1527–1529
13. Weber B, Welte M, Hammer C, et al (1984) Increase of natural killer cells in rejecting kidney grafts. Transplant Proc 16: 1177–1178
14. Whiteside TL, Wilson J, Bryant J, Zeevi A, Abu el-Magd K, Jain A, Alessiani M, Fung J, Herberman RB, Starzl TE (1991) Serial monitoring of immunologic function and phenotype of lymphocytes in the blood of transplanted patients randomized to cyclosporine or FK506. Transplant Proc 23: 3047–3051