Immunophenotypic Pattern of De Novo Malignancy After Liver Transplantation

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ABSTRACT

Long-term survival after liver transplantation is affected by de novo neoplasia. The incidence of this type of malignancy is increased in the setting of immunosuppressive therapy. The aim of this study was to characterize the immunologic pattern of liver transplant recipients with de novo malignancies. Fifty-one liver recipients were studied, 19 of whom had a history of de novo neoplasia. Immunophenotypic patterns among patients with/without tumors were compared. The subpopulations of $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes differed between the 2 types of patients studied. In patients with tumor, activation membrane markers in $CD4^+$ T lymphocytes and $CD8^+$ T-lymphocytes, such as CD56 or CD25, were expressed in a greater proportion, whereas activation markers CD314 and CD16 were reduced in $CD56^{bright}$ natural killer (NK) cells. We concluded that cytotoxic response seems to be more activated in de novo neoplasia patients, which highlights the still unknown malignancy risk effect on these immune cells.

LONG-TERM survival after liver transplantation is conditioned by the risk of de novo neoplasia [1–3]. To extend survival in these patients, a detailed study of risk factors for developing malignancies should be ruled out. After transplantation, patients have at least a 2-fold risk of cancer compared with the general population, which may be related to the effects on immune cells by drugs used to control graft rejection [4–6]. Loss of immune surveillance because of immunosuppressive therapy may increase neoplasia incidence [7], but the mechanisms of this increased risk are not clear. The aim of this study was to determine whether there are differences in immunologic patterns of patients with/without posttransplant tumors.

MATERIALS AND METHODS

Patients >18 years old with at least 1 year of follow-up were selected after liver transplantation at the Clínica Universidad de Navarra (Pamplona, Spain). Patients were excluded from study participation if they were receiving drugs that could also affect the immune system, apart from their immunosuppressive therapy, or if they had discontinued graft antirejection treatment.

Flow cytometry was performed using a cytometer (FACS Canto II; Becton-Dickinson) and FACSDiva version 6.0 software. The quantities of myeloid and plasmocytoid dendritic cells, $CD4^+$ and $CD8^+$ T-lymphocyte subpopulations (naive, central memory, effector, and memory effector), and natural killer (NK) cells ($CD56^{bright}$, canonical $CD56^{dim}$, and adaptive $CD56^{dim}$) were measured using membrane marker antibodies as reagents, and expressed as percentages. Their respective membrane marker expression levels were also examined in the same assay measuring the mean fluorescence intensity (MFI) of each marker in each cell subpopulation. Quantification of the different populations and expression levels of these markers were analyzed with Infinicyt software version 1.8 (Cytognos) and were also compared between transplanted patients with/without malignancies.

In this cross-sectional study, categorical variables were described using frequency and percentage data. Median and interquartile interval data were used to describe quantitative variables. Comparisons of the quantity of cells or the levels of expression for each membrane marker were made using the Mann-Whitney *U* test, Student's *t* test, or Welch's test, in accordance with distribution of

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Table 1. Clinical Features of Patients With De Novo Neoplasia and Patients Without Tumor

	Patients With De Novo Neoplasia	Patients Without De Novo Neoplasia	*P* Value
Age at liver transplantation	55.42 ± 4.3	52.26 ± 7.5	.06
Sex			
Men	18 (94.7%)	26 (83.9%)	.38
Women	1 (5.3%)	5 (16.1%)	.38
BMI (kg/m^2)	26.96 ± 3.0	27.28 ± 3.3	.77
Original disease			
Alcoholic cirrhosis	12 (63.2%)	15 (48.4%)	.30
HVC cirrhosis	4 (21.1%)	7 (22.6%)	1.00
Others	3 (15.8%)	9 (29.0%)	.33
Previous hepatocellular carcinoma	9 (47.4%)	11 (35.5%)	.40
Immunosuppressive treatment			
Tacrolimus	8 (42.1%)	23 (74.2%)	.02
Cyclosporine	5 (26.3%)	4 (12.9%)	.27
Mycophenolate mofetil	10 (52.6%)	21 (67.7%)	.28
Prednisone	3 (15.8%)	2 (6.5%)	.62
Everolimus	2 (10.5%)	1 (3.2%)	.14

Data expressed as mean ± SD or as number (%).
Abbreviations: BMI, body mass index; HCV, hepatitis C virus.

the variables. Statistical analysis was performed using SPSS version 20.0 software (IBM, Inc).

RESULTS

Samples from 51 patients were collected. The majority were men (86%), age (mean ± SD) 54 ± 6.6 years. In 56.9% of patients, the reason for transplant was alcoholic cirrhosis. Forty-six patients (90.2%) received cadaver donor liver transplantation, whereas 9.8% (n = 5) of the patients received living donor transplantation. Nineteen patients (37.2%) had a posttransplant malignancy and only 1 of these patients was a living donor recipient. Median time from transplant to diagnosis of de novo neoplasia was 56 (interquartile range [IQR], 34–108) months. There were differences between patients who developed de novo neoplasia and those who did not, as shown in Table 1.

Table 2 shows findings on number of immune cells in patients who did and did not develop malignancy.

Levels of expression of membrane markers in patients with/without de novo malignancies were also compared. In T lymphocytes from patients with tumors, CD56 membrane marker was proportionally greater (median, 10.47 [IQR, 8.8–15.6]; $P = .03$). $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes expressed less CD25 (median, 48.68 [IQR, 39.8–61.7]; $P = .01$) and less CD279 (median, 29.82 [IQR, 24.1–44.6]; $P = .03$), respectively.

DISCUSSION

There is currently enough evidence to affirm that T lymphocytes, especially $CD8^+$ T lymphocytes, play a fundamental role in surveillance and defense against tumors. Previous studies on kidney and hepatic transplant recipients who developed de novo malignancies suggested a different immunophenotypic pattern between the groups [8,9].

In our study we did not find significant differences in the quantity of T lymphocytes in patients with neoplasia compared with nontumor patients. However, subpopulations of $CD4^+$ T lymphocytes and $CD8^+$ T lymphocytes differed in patients with/without cancer, as shown in Table 1. This may be part of the inherent immunologic response against neoplastic cells or a compensation mechanism due to the inability of these cells to correctly perform their function. The exact role of the tumor's microenvironment over immune cells is still not entirely clear. It has

Table 2. Quantities of Immune Cells of Patients With/Without De Novo Neoplasia

	Patients With De Novo Neoplasia	Patients Without De Novo Neoplasia	*P* Value
NK cells	2.3 (1.8–4.4)	2.4 (1.5–4)	.87
$CD56^{bright}$	3.5 (2.1–7.2)	4.2 (2.8–9.8)	.23
Canonical $CD56^{dim}$	17.8 (11.9–31.8)	23.9 (17.5–31.4)	.30
Adaptive $CD56^{dim}$	73.5 (65.3–82.4)	63.3 (56.7–76.3)	.09
$CD4^+$ T lymphocyte	52.6 (43.5–60.2)	55.4 (47.4–61.7)	.44
Naive	25.4 (11.4–41.7)	21.5 (11.6–33.3)	.60
Central memory	41.6 (32.1–49.3)	48.9 (36.2–61.1)	.10
Effector	2.76 (0.4–5.7)	2 (0.5–5.8)	.89
Memory effector	22.3 (15.3–32.3)	18.3 (12.6–24.3)	.12
$CD8^+$ T lymphocyte	39.4 (33.7–47.6)	35.5 (29.9–39.8)	.22
Naive	11.7 (5.0–15.4)	10.5 (5.9–17.0)	.98
Central memory	9.6 (4.4–13.8)	12.3 (6.6–25.0)	.07
Effector	52.7 (29.3–59.7)	41.9 (27.6–54.6)	.74
Memory effector	26.6 (18.0–38.9)	18.7 (10.5–38.7)	.10
Dendritic cells			
Myeloid	0.16 (0.11–0.22)	0.17 (0.12–0.24)	.54
Plasmocytoid	0.13 (0.09–0.15)	0.14 (0.09–0.18)	.53

All values are expressed in median of cell percentage and interquartile range.
Abbreviation: NK, natural killer.

been postulated that the microenvironment favors the tumor's tolerance by inducing anergy, senescence, or destruction of the immune cells [10]. Yet, the exact function of effector-memory $CD4^+$ T lymphocytes in cancer is still unknown, as they also have much cytotoxic ability, which is affected by the tumor's microenvironment and interfered with by immunosuppressive medication [11].

Assessment of membrane markers in T lymphocytes could provide valuable information on their maturity or activation state. In our sample, patients who developed de novo neoplasia demonstrated a tendency toward increased expression of CD57 (median, 25.73 [IQR, 14.9–31.2]; $P = .33$). They also showed statistically significant greater expression of CD56 and decreased expression of CD25 in $CD4^+$ T lymphocytes and of CD279 in $CD8^+$ T lymphocytes. Thus, it is plausible to believe that, although the number of total T lymphocytes remains constant, there is a shift toward a $CD8^+$ T-lymphocyte cytotoxic response. Indeed, these data seem to indicate that, even though T lymphocytes of tumor patients appear to be more activated, they could be in a senescent state or have impaired cytotoxic capacity.

We did not find relevant immunophenotypic differences regarding regulatory T cells or gamma-delta T cells, in contrast to the available evidence suggesting a relationship with risk of neoplasia.

Evidence about the role of NK cells in antitumor immunosurveillance is subject to debate. The proportion of $CD56^{dim}/CD56^{bright}$ NK cells could help to explain shed some light on this issue, as $CD56^{dim}$ NK cells are known to be more cytotoxic. In this study, we detected no differences in NK-cell membrane markers. Nonetheless, larger samples will be needed to affirm and appreciate the differences between these groups.

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