Bull. Yamaguchi Med. Sch., Vol. 28, Nos. 1-2, 1981

The Host's Cellular Immune Status and Therapy for Liver Cancer

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(Received November 28, 1980)

Abstract. The skin reactions to purified protein derivative (PPD), phytohemagglutinin (PHA) and polysacchride antigens from streptococcal hemolyticus (PS-E), were depressed in the initial stage of the patients and markedly depressed in the end stage. The number of T-cells in peripheral blood decreased with progress of illness, but the number of PHA-blastoid cells and active T-cells increased sometimes in the end stage. It suggests that these factors as well as skin tests are useful parameters for evaluation of the cellular immune status of the patients with primary liver carcinoma. The host's immune status was impaired frequently either by the disease itself or by chemotherapy. In cases receiving chemotherapy in combination with the administration of OK-432, the depressed skin reaction to PS-E improved often. These findings suggest that the administration of OK-432 affect the host and that result of this skin test is useful as a parameter to determine the method and period of OK-432 administration and to judge its effectiveness.

Key Words: primary carcinoma, cellular immune status, chemotherapy, skin test, immune parameter

Introduction

It is well known that anticancer drugs have a depressive effect on the bone marrow and immune organs other than the intrinsic action to inhibit the growth of tumor cells [1,2]. Therefore, administration of anticancer drugs should be preferable made in view of the host's immune status. To grasp the host's cellular immune status and to improve the depressed immune status in the patients with cancer will be helpful for promotion of the host's immunological surveillance.

Recently, immunotherapy against human carcinoma has progressed[3], and success of immunotherapy is expected also in the treatment of liver carcinoma. At present, however, there are very few studies even on the cellular immune status of patients with liver carcinoma. We have studied the cellular immune status of patients with liver carcinoma[4-8]. In this paper, we report the clinical significance of immunological monitoring tests, especially in relation to therapy.

Materials and Methods

1. Subjects

Subjects are patients who were admitted to the Department of Internal Medicine, Yamaguchi University School of Medicine during the period from 1973 to 1978 and diagnosed histologically as hepa-

tocelluar carcinoma (PHC) and a few patients with metastatic liver carcinoma (MLC).

The effect of chemotherapy on cellular immune parameters was studied in 17 cases. These cases were divided into two groups. the chemotherapy group of 8 cases with an average age of 63 years old (5 males and 3 females including 4 cases of MLC) and the immunochemotherapy group with an average age of 52 years old (6 males and 3 females including 5 cases of MLC). OK-432 which was made from streptococcal haemolyticus by Okamoto et al[9] was used as a immunopotentiator in the present study. OK-432 was given intramuscularly 2 or 3 times a week at the mean average of 2.7 KE/week.

2. Cellular immune parameters

1) Skin tests.

Skin tests before and after the chemo- or immuno-chemotherapies were performed at intervals of 62 days on an average in the former group and of 45 days on an average in the latter group. The following antigens were injected subcutaneously in a dose of 0.1 ml each to the forearm of a patients.

The reaction to PPD was judged 48 hours after injection and that to PHA and PS-E, 24 hours after injection.

a) PPD : purified protein derivative (Japan BCG Lab.), 0.08μg/0.1ml

b) PHA : Phytohemagglutinin (Welcome Co.), 5μg/0.1ml

c) PS-E : polysacchride antigens extracted from streptococcal hemolyticus (Su-strain) by the procedure as reported by Slade[10], 5μg/0.1 ml.

Table 1 Grade of skin test to antigen

Grade	Erythema (mm)
0	0-4
1	5-9
2	10-14
3	15-19
4	20-29
5	30-39
6	over 40

In judgment of skin tests, the size of reactive erythema was graded according to the length of its longest diameter[11] as shown in Table 1. In case of erythema of Grade 0 or 1, skin tests were juged to be negative, and in case of erythema of and over Grade 2, to be positive.

2) Count and subsets of peripheral lymphocytes

The count of peripheral lymphocytes was obtainted from the laboratory data. T-cells were counted using the sheep red blood cell rosette formation method, and B-cells, using indirect immunofluorescence[6]. Active T-cells were counted by Wybran's method[12]. PHA stimulation was performed in the following method. Lymphocytes were suspended in TC-199 containing 20% bovine serum in the ratio of $1\text{-}2\times10^6$ cells/ml, 1μg of PHA-P (10μg/ml,

Table 2 Immune parameters in patients with hepatocellular carcinoma

		Normal control	Patients	
			Initial stage	End stage
Skin tests to (%of positive)	PPD	89 (28)	54 (22)	25 (16)
	PHA	86 (22)	36 (22)	18 (16)
	PS-E	82 (12)	54 (11)	12 (16)
		mean±SD		
Lymphocytes (mm^3)		800-4,000	1,231±142(44)	632 ±42 (51)
PHA-blastoid cells (%)		56 ±3 (20)	34 ±2 (38)	44 ±3 (10)
T-cells (E-rosette %)		62 ±3 (36)	48 ±2 (39)	41 ±4 (24)
Active T-cells (%)		28 ±2 (12)	22 ±2 (21)	19 ±2 (16)
B-cells (%)		27 ±2 (10)	29 ±2 (21)	31 ±3 (14)

( ) : Number of patients tested

Difco, Detroit) was added, the suspension was incubated in a 5% CO_2 incubator at 37°C for 3 days, and then the percentage of PHA blastoid cells in the suspension was counted[6,13].

Results

1. Cellular immune status of patients with liver carcinoma

Table 2 shows various cellular immune status in the initial stage and in the end stage (for one month before death) in comparison with that in healthy subjects. The rates of positive reaction to various skin tests in healthy subjects were 89.2% to PPD, 86.3% to PHA and 82.3% to PS-E, but in patients with liver carcinoma in the initial stage, those were 54.5%, 36.3% and 54.5%, respectively. In the end stage of the patients, the positive rates of skin tests were 25.0% to PPD, 18.7% to PHA and 12.5% to PS-E. Thus, the results of these skin tests suggest that cellular immune status of patients with liver carcinoma have been depressed already in the initial stage and more remarkably in the end stage.

The peripheral lymphocyte count was $1,231 \pm 142/mm^3$ in the initial stage and decreased significantly to $632 \pm 42/mm^3$ in the end stage.

The percentages of PHA-blastoid cells and T-cells in the peripheral lymphocytes were $56 \pm 3\%$, $62 \pm 3\%$ respectively in healthy subjects, but those in patients with initial stage of liver carcinoma were $34 \pm 3\%$, $48 \pm 2\%$. In advanced stage of patients, the percentage of T-cells further decreased. The percentage of active T-cells in healthy subjects was $28 \pm 3\%$ of peripheral lymphocytes, but that was $22 \pm 2\%$ in patients with liver carcinoma in the initial stage and decreased to $19 \pm 2\%$ in the end stage. The percentage of B-cells in healthy subjects was $27 \pm 2\%$ of peripheral lymphocytes, but that was $29 \pm 3\%$ in patients with liver carcinoma in the initial stage, and increased to $31 \pm 3\%$ in the end stage. These results show a slight rise in the count of B-cells and a relative fall in the count of T-cells in the peripheral blood of patients with liver carcinoma.

2. Effect of therapy on various skin tests

Fig. 1 shows the changes in grade of skin tests in chemotherapy and immunochemotherapy groups before and after treatments. In most cases of the former group, the grades of various skin tests decreased or remained unchanged following the therapy.

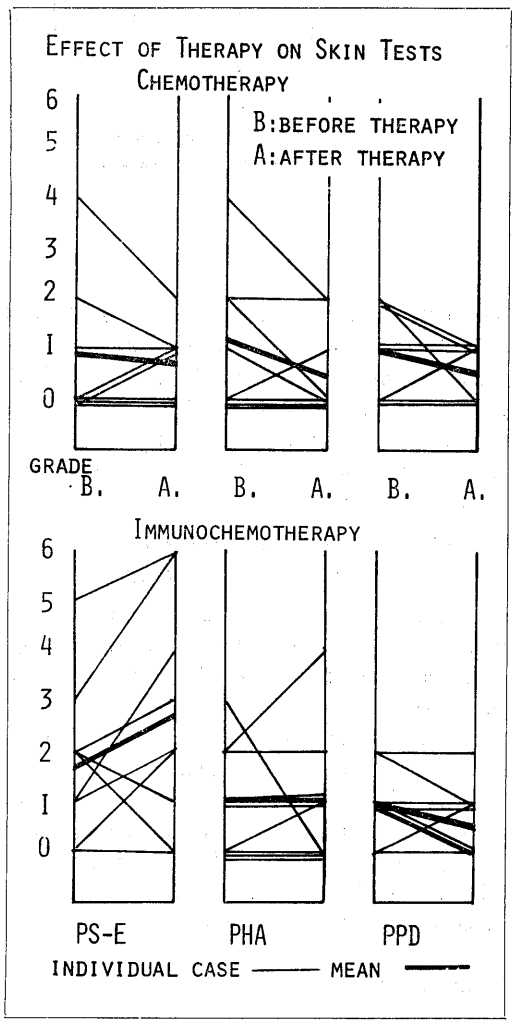

Fig. 1

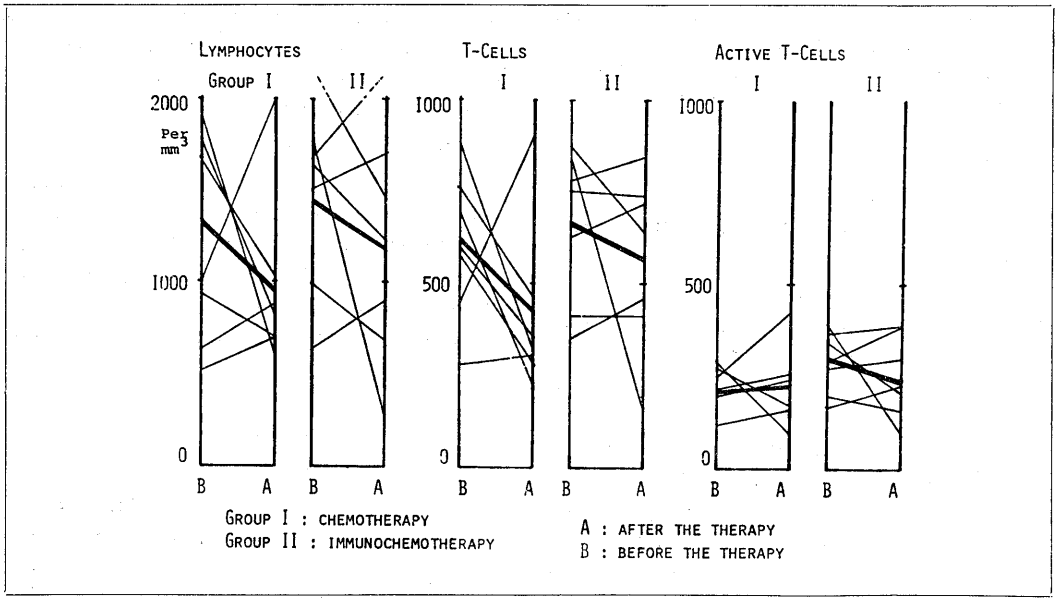

Fig. 2

The latter group, on the other hand, showed a slight increase in the grade of skin tests after the therapy; that is, 6 cases to PS-E, two to PHA and one to PPD. The mean grade of PS-E skin test was 1.7 cm before therapy, but increased to 2.6 cm after immunochemotherapy.

3. Effect of therapy on the peripheral lymphocyte count and the T-cell subsets.

Fig. 2 shows the changes in peripheral lymphocyte count and T-cell subsets in the same patients. In both chemotherapy and immunochemotherapy groups, lymphocyte count decreased and significantly decreased from $1,337 \pm 471$ to $938 \pm 522/mm^3$ ($P \leqq 0.05$) in the chemotherapy group. T-cell count decreased significantly from 601 ± 180 to $404 \pm 231/mm^3$ ($P \leqq 0.05$) after chemotherapy. The active T-cell count remained unchanged or increased slightly in both groups.

Discussion

It has long been known that the positive rate in the skin test to PPD is low in the cancer patients[14]. There are a few studies on the results of various skin tests in patients with liver carcinoma, but as shown by this study, the positive rate in case of liver carcimona was as low as in case of cancer of the gastrointestinal tract including gastric carcinoma[13]. It is noteworthy that the positive rate in the skin test in patients with advanced cancer was remarkably low. The results are similar to those reported by Nemoto et al. from skin tests in patients with advanced lung cancer[15]. Those findings indicate that the depression of cellular immune status as shown in the delayed-type skin test is specially remarkable in patients with advanced cancer and that skin tests to various antigens appear to be clinically useful in such a way that the total cellular immune status can be simply estimated also in patients with liver carcinoma. In addition, these tests are expected to be used as the monitor for treatment of liver carcinoma.

In the present study, however, there are

few cases showing improvement in skin tests to PPD and PHA after chemotherapy and even after immunochemotherapy, in contrast to the results in patients with gastric carcinoma[16]. This may have been due to immunodeficiency already existing in most of our cases, which consisted predominantly of advanced carcinoma. Therafter various treatments may have had no influence on the results of skin tests. Furthermore, this phenomenon may be attributed to the larger tumor load in patients with liver carcinoma than those with other carcinoma.

The counts of peripheral lymphocytes and T-cells in patients with liver carcinoma were decreased after chemotherapy and the decrease of the latter was significant. The cause of such a decrease in T-cell count has not yet completely elucidated, but the suppressive effect of antitumor agents on the immune organs[1,2] is considered as one of the factors.

In recent years, the active T-cell has attracted our interest as a specific subsets of T-cell[12,17]. In the present study, slight decrease of active T-cell was observed in the initial stage of liver carcinoma, comparing with that of normal subjects. The active T-cell count as well as PHA-blastoid cell count remained unchange or slightly increased after treatment in both groups. Further observations are necessary for understanding relationship between the host's cellular immune status and chemotherapy of cancer patients.

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