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GAMMA INTERFERON PRODUCTION BY PERIPHERAL BLOOD LYMPHOCYTES IN PATIENTS WITH GASTRIC CANCER

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ABSTRACT

The production of gamma interferon (IFN-γ) by peripheral blood lymphocytes was studied in 38 patients with gastric cancer. Purified protein derivative (PPD) as a specific antigen and three kinds of mitogens, phytohemagglutinin-P (PHA), concanavalin A (Con A) and pokeweed mitogen (PWM), were used as IFN-γ inducers. IFN-γ production by lymphocytes was markedly reduced in patients with gastric cancer when these inducers were used. Reduction of IFN-γ production was observed more often in patients of Stage IV than in those of Stage I when PPD or Con A was used. PPD-induced IFN-γ production significantly increased in patients who were operated on curatively, while it decreased in those who were operated on noncuratively. IFN-γ production by lymphocytes, especially when PPD is used as an IFN-γ inducer, reflects cell-mediated immunity in patients with gastric cancer. It may be useful as quantitative parameter to predict therapeutic effects and prognosis in cancer patients.

Key words: Gamma Interferon, Gastric Cancer, Cell-mediated Immunity, Quantitative Parameter

INTRODUCTION

A number of parameters, such as delayed hypersensitivity skin test, the ratio of T lymphocytes (T cells) and blastogenic response of lymphocytes to certain mitogens, have been studied concerning cell-mediated immunity in patients with malignancy. These parameters have been used to estimate the therapeutic effects and prognosis in those patients. Furthermore, lymphokines, which originate from lymphocytes as mediators of cell-mediated immunity, have been measured, and macrophage migration inhibitory factor (MIF) has been assayed as a representative parameter of lymphokine production.[1,2]

Interferon (IFN) has been demonstrated to be produced on immune-specific bases.[3,4] This type of IFN has been called immune IFN,[5] now named IFN-γ, and is regarded as one of the lymphokines. It is separated from alpha IFN (IFN-α) or beta IFN (IFN-β) according to antigenic and physico-chemical properties. Titration of IFN-γ has shown that it may excel MIF in quantitative analysis of lymphokine production. The present study is designed to examine whether IFN-γ production by lymphocytes is useful parameter of cell-mediated immunity in patients with gastric cancer.

MATERIALS AND METHODS

Subjects

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Thirty-eight untreated patients with gastric cancer were studied. The patient group consisted of 26 males aged 49 to 78 and 12 females aged 37 to 71. Patients were divided into four groups: Stage I (14 patients), Stage II (4 patients), Stage III (6 patients) and Stage IV (14 patients) according to the definition of the Japanese Research Society for Gastric Cancer. The patients of Stage II and Stage III were combined into one group in this study. The control subjects were composed of 17 males aged 31 to 85 and 7 females aged 39 to 68 (Table 1).

Table 1 Subjects

	Number	Male	Female	Age
Control	24	17	7	61.4±2.7
Gastric cancer	38	26	12	61.8±1.6
Stage I	14	8	6	57.6±2.7
Stage II,III	10	6	4	61.3±3.5
Stage IV	14	12	2	66.3±1.9

Lymphocyte culture

Fifteen ml of heparinized peripheral blood were obtained from each patient with gastric cancer and from each control subject. Lymphocytes were isolated by sedimentation in Ficoll-Paque (Pharmacia, Uppsala, Sweden). They were suspended at a concentration of 1×10^6 cells per ml in RPMI 1640 supplemented with 10% fetal calf serum. PPD (Mitsui, Tokyo, Japan), PHA (Difco, Detroit, U.S.A.), Con A (Miles, Elkhart, U.S.A.) and PWM (Gibco, Grand Island, U.S.A.) were added to the culture medium. The final concentration of PPD was 5 μg/ml and that of PHA, Con A and PWM was 50 μg/ml. Lymphocytes were cultured in the above medium at 37°C in a humidified atmosphere containing 5% CO_2, and the peak titers of IFN-γ in the supernatants were observed from the third to the seventh day. Some Lymphocytes were cultured in medium alone as a control. On the fifth day of the culture in all cases, cells were centrifuged and the supernatants were obtained. The supernatants were stored at −20°C until IFN assay.

IFN assay

IFN activity was measured by observing inhibition of the cytopathic effect (CPE) of vesicular stomatitis virus (VSV) on human amnion FL cells.[6] IFN titer (U/ml) was defined as the reciprocal of the last dilution to inhibit 50% CPE on FL cells. IFN activity was expressed as $\log_2$ IFN titer. Each sample was titrated in duplicate.

Antiviral activity was not recognized when mouse L cells were used instead of FL cells. Moreover, inhibition of CPE on FL cells was observed when Sindvis virus was used in place of VSV. Antiviral activity was completely lost when dialyzed against glycine HCl buffer (pH 2) for 24 hours. Treatment of the supernatants with anti-IFN-α and anti-IFN-β antibodies showed no reduction in IFN titer. The antiviral activity in the supernatants was considered to be IFN-γ.

Ratio of T cells in peripheral blood lymphocytes

Lymphocytes were incubated with twenty-fold sheep erythrocytes at 4°C for 12 hours. After staining of lymphocytes by brilliant cresyl blue, the ratio of T cells was assayed by counting the number of rosette-shaped cells with sheep erythrocytes.[7,8]

Blastogenic response of lymphocytes

Blastogenic response of lymphocytes was assayed by measuring the incorporation of

^{3}H-thymidine into deoxyribonucleic acid in 1×10^5 lymphocytes for the final 8 hours of the culture period.[9]

PPD skin reaction

Delayed hypersensitivity was tested by intradermal injection of tuberculin. Erythema of 1.0 cm or greater in diameter at 48 hours was defined as positive.

Statistical analysis

All data were expressed as mean ± SE. The significance of differences between mean values was analyzed by using t-test. Linear regressions were determined by the method of least mean squares. Significance was accepted at the 5% level.

RESULTS

Lymphocyte count

Lymphocyte count was 1,870 ± 110 / cmm in the patients with gastric cancer and 2,230 ± 130 / cmm in the control subjects, showing a reduction of lymphocytes in the cancer patients ($p < 0.05$).

Ratio of T cells

Lymphocytes from 27 patients with gastric cancer contained 53.0 ± 1.9% T cells, while those from 11 control subjects contained 60.3 ± 2.0% T cells. The ratio of T cells, was reduced in the cancer patients ($p < 0.05$).

Blastogenic response of lymphocytes

Blastogenic response of lymphocytes was measured in 13 patients with gastric cancer and in 10 control subjects. Incorporation of ^{3}H-thymidine was 36,900 ± 4,800 cpm in the cancer patients and 35,400 ± 4,900 cpm in the control subjects, when PHA was added to the lymphocytes, while it was 30,200 ± 3,600 cpm and 33,400 ± 3,600 cpm, respectively, when Con A was added to the lymphocytes. The difference of blastogenic response of lymphocytes between the two groups was not statistically significant.

IFN-γ production by lymphocytes

IFN-γ activity in the supernatants of lymphocytes cultured in medium alone was 2.6 ± 0.1 in 38 patients with gastric cancer and 2.3 ± 0.2 in 24 control subjects. There was no significant difference between the two groups.

IFN-γ activity in the supernatants of lymphocytes cultured with PPD was 4.0 ± 0.2 in the patients with gastric cancer and 5.5 ± 0.4 in the control subjects. IFN-γ production was reduced in the cancer patients ($p < 0.001$). Analysis among the cancer patients revealed that IFN-γ production was more greatly reduced in patients of Stage IV compared with those of Stage I ($p < 0.05$) (Table 2).

IFN-γ activity in the supernatants of lymphocytes cultured with PHA was 5.1 ± 0.2 in the patients with gastric cancer and 6.5 ± 0.3 in the control subjects. IFN-γ production was reduced in the cancer patients ($p < 0.001$). There was no significant difference of IFN-γ production among the different clinical stages of the disease (Table 3).

When lymphocytes were cultured with Con A, IFN-γ activity in the supernatants was 4.3 ± 0.2 in the patients with gastric cancer and 5.9 ± 0.2 in the control subjects. IFN-γ production was reduced in the cancer patients ($p < 0.001$). Analysis among the cancer patients revealed that IFN-γ production was reduced in the patients of Stage IV compared with those of Stage I ($p < 0.05$) (Table 4).

When lymphocytes were cultured with PWM, IFN-γ activity in the supernatants was 5.4 ± 0.2 in the patients with gastric cancer and 6.3 ± 0.3 in the control subjects. IFN-γ production was reduced in the cancer patients ($p < 0.01$). There was no significant difference

of IFN-γ production among the varying stages of the disease (Table 5).

IFN-γ production was analyzed according to the histological findings of gastric cancer. There was no significant difference of IFN-γ production between the group of 18 patients with well-differentiated carcinoma and that of 20 patients with poorly-differentiated carcinoma.

IFN-γ production and ratio of T cells

There was no significant correlation between IFN-γ activity in the supernatants and the ratio of T cells in the patients with gastric cancer ($r = 0.14$ for PPD, $r = 0.24$ for PHA, $r = 0.13$ for Con A and $r = 0.03$ for PWM) and in the control subjects ($r = -0.09$ for PPD, $r = 0.34$ for PHA, $r = 0.04$ for Con A and $r = -0.17$ for PWM).

IFN-γ production and blastogenic response of lymphocytes

IFN-γ activity in the supernatants did not correlate with blastogenic response of lymphocytes either in the patients with gastric cancer ($r = 0.29$ for PHA and $r = 0.21$ for Con A) or in the control subjects ($r = 0.40$ for PHA and $r = 0.36$ for Con A).

IFN-γ production and PPD skin reaction

Twenty-two patients with gastric cancer and 18 control subjects underwent PPD skin test. Twelve patients were positive and 10 patients were negative. The control subjects were all positive for PPD skin test. When lymphocytes were cultured with PPD, IFN-γ activity in the supernatants in the patients with positive reaction was 4.6 ± 0.3, which was higher than the value of 2.9 ± 0.4 in the patients with negative reaction ($p < 0.005$). However, it was lower than the value of 6.0 ± 0.4 in the control subjects with positive reaction ($p < 0.05$) (Table 6).

Table 2 IFN-γ activity in the supernatants of lymphocytes cultured with PPD

	Number	IFN-γ activity (log_2 IFN-γ titer)
Control	24	5.5±0.4
Gastric cancer	37	4.0±0.2
Stage I	14	4.6±0.3
Stage II, III	10	3.9±0.5
Stage IV	13	3.5±0.4

Table 3 IFN-γ activity in the supernatants of lymphocytes cultured with PHA

	Number	IFN-γ activity (log_2 IFN-γ titer)
Control	24	6.5±0.3
Gastric cancer	35	5.1±0.2
Stage I	12	5.4±0.3
Stage II, III	10	4.6±0.4
Stage IV	13	5.1±0.4

Table 4 IFN-γ activity in the supernatants of lymphocytes cultured with Con A

	Number	IFN-γ activity (log_2 IFN-γ titer)
Control	24	5.9±0.2
Gastric cancer	35	4.3±0.2
Stage I	12	5.0±0.4
Stage II, III	10	4.3±0.4
Stage IV	13	3.8±0.4

Table 5 IFN-γ activity in the supernatants of lymphocytes cultured with PWM

	Number	IFN-γ activity (log_2 IFN-γ titer)
Control	24	6.3±0.3
Gastric cancer	37	5.4±0.2
Stage I	13	5.6±0.3
Stage II, III	10	4.9±0.4
Stage IV	14	5.5±0.3

Table 6 Relationship between PPD skin reaction and PPD-induced IFN-γ production

	Skin reaction	Number	IFN-γ activity (log_2 IFN-γ titer)
Control	positive	18	6.0±0.4
Gastric cancer	positive	12	4.6±0.3
	negative	10	2.9±0.4

Changes of IFN-γ production following operation

IFN-γ production by lymphocytes was evaluated from one to four months after operation in the patients with gastric cancer who had not received either chemotherapy or immunotherapy. In 5 patients who were operated on curatively, PPD-induced IFN-γ production increased after operation ($p < 0.05$). IFN-γ production induced by other mitogens also increased in most patients. In contrast, in 6 patients who were operated on noncuratively, PPD-induced IFN-γ production decreased after operation ($p < 0.05$). IFN-γ production induced by other mitogens also decreased in most patients (Fig. 1).

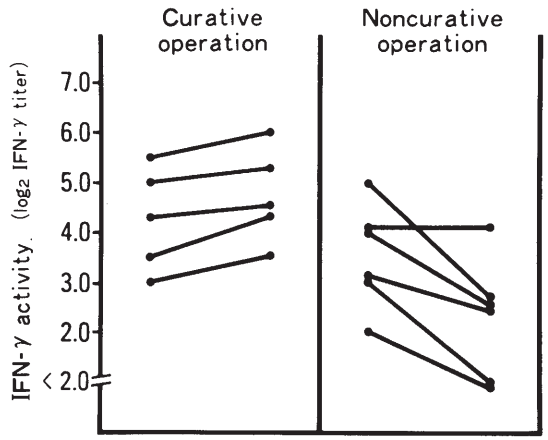

Fig. 1 Changes of PPD-induced IFN-γ production following operation

DISCUSSION

There have been some investigations concerning IFN production by lymphocytes in patients with malignancy;[10~14] IFN production, however, has not been thoroughly studied in patients with solid malignant tumors. Wakui *et al*[13] studied IFN production induced by PHA in patients with advanced cancer. IFN production was reduced in those patients. On the other hand, in studies performed by Kadisch *et al*[14] IFN production induced by Newcastle disease virus was normal in cancer patients in all clinical stages of the disease. Discrepancies between these observations may be due to the difference of IFN inducers. IFN-γ is produced by lymphocytes when antigens or mitogens are used as IFN inducers, while IFN-α is produced when viruses are used. We studied IFN-γ production by lymphocytes, which is considered to reflect cell-mediated immunity more specifically, and demonstrated that IFN-γ production by lymphocytes was significantly reduced in patients with gastric cancer.

Our data concerning stages of the disease indicated that IFN-γ production was significantly reduced in patients of Stage IV as compared with those of Stage I when PPD was used as an IFN-γ inducer. On the other hand, IFN-γ production was not always reduced when mitogens were used as IFN-γ inducers. As PPD is considered to be a specific antigen, PPD-induced IFN-γ production seems to reflect cell-mediated immunity more specifically.

IFN-γ is considered to originate mainly from T cells.[15,16] It may be of interest as to whether lowered IFN-γ production by lymphocytes is caused by decrease of T cells or by their functional deficiency in patients with gastric cancer. There was no correlation between IFN-γ production and the ratio of T cells, although the ratio of T cells was reduced in cancer patients. These data suggest that lowered IFN-γ production in cancer patients may be due to functional deficiency of T cells. However, further studies on the role of macrophages are required, because the production of IFN-γ needs the interaction of lymphocytes and macrophages.[17]

IFN-γ production did not correlate with blastogenic response of lymphocytes in cancer patients and control subjects, when PHA and Con A were used as inducers. These observations parallel the work of Epstein *et al*[17] and Wallen *et al.*[18] IFN-γ production may be a different parameter to assess functions of lymphocytes in cancer patients.

PPD skin reaction is considered to be a representative parameter of cell-mediated immunity in vivo. PPD-induced IFN-γ production by lymphocytes was significantly reduced in cancer patients with positive skin reaction compared with control subjects with positive reaction. These results seem to be due to impaired function of T cells in cancer patients. In vitro IFN-γ production can be used as a more quantitative parameter to evaluate cell-mediated immunity in cancer patients.

PPD-induced IFN-γ production significantly increased after operation in patients who were operated on curatively. IFN-γ production induced by other mitogens also increased in most patients. In contrast, PPD-induced IFN-γ production significantly decreased in those patients who were operated on noncuratively. IFN-γ production induced by other mitogens also decreased in most patients. These observations agree with the work of Wakui *et al*[13] on the changes of PHA-induced IFN-γ production after chemotherapy, suggesting that IFN-γ production by lymphocytes may help predict the therapeutic effects in cancer patients.

IFN has been demonstrated to affect various cellular functions including induction of cytotoxic T cells,[19] activation of macrophages[20] and enhancement of natural killer cells.[21] Assessment of IFN-γ production by lymphocytes reflects cell-mediated immunity and may be useful in predicting the therapeutic effects and prognosis in cancer patients.

CONCLUSION

IFN-γ production by lymphocytes was markedly reduced in patients with gastric cancer when PPD and mitogens were used as inducers. IFN-γ production was reduced in patients of Stage IV as compared with those of Stage I when PPD or Con A was used. PPD-induced IFN-γ production significantly increased in patients who were operated on curatively, while it decreased in those who were operated on noncuratively.

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