Blastogenic Response of Lymphocytes from Patients with Cancer of the Uterine Cervix

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ABSTRACT

The cellular immune status of patients with cancer of the uterine cervix was investigated by estimating lymphocyte response to phytohemagglutinine. The isolated lymphocyte culture of cancer patients did not show depressed proliferative response. On the other hand, the whole blood culture of even patients with early stages showed an apparently depressed response and the clinical stages coincided with the degree of the response. When the plasma components were replaced by the medium in the blood from patients with cervical cancer, the responses to the agglutinin of lymphocytes increased, suggesting that the autologous serum in the whole blood culture might play a role in reducing the proliferative responses of lymphocytes.

Key words: blastogenic response; autologous serum; cervical cancer

INTRODUCTION

Transformation of human peripheral lymphocytes to large "blast" forms was first noted in cultures containing phytohemagglutinine (PHA) [1]. It was soon discovered that this response also occured when lymphocytes from patients with cutaneous delayed hypersensitivity were exposed to the same antigens in culture[2]. Then, lymphocyte responses to PHA have been studied as an in vitro test of delayed hypersensitivity in various kinds of malignant diseases. Generally, two kinds of methods have been employed for clinical purposes; the isolated lymphocyte culture (ILC) method and the whole blood culture (WBC) method. As for cervical cancer, Yamagata and Green[3] reported that, with WBC, both early and advanced stages of the disease showed suppressed lymphocyte responses. On the other hand, Hagen et al[4]. stated that, with ILC, patients with non-metastatic cervical cancer did not show any depression of lymphocyte responses. It is interesting to note that there appear

to be some discrepancies between the data obtained by these two methods about cervical cancer. The present study was designed to investigate these contradictory results by employing these two tests at the same patients with cervical cancer. Attempts were also made to clarify these discrepancies, by studying the effect of autologous serum on lymphocyte responses in WBC.

MATERIALS AND METHODS

Patients. Sixty patients with cancer of the uterine cervix whose ages ranged from 27 to 74 yr were studied. Cancer patients were staged according to the FIGO (international federation of gynecology and obstetrics) classification. The control group consisted of 21 patients with myoma of the uterus whose age distribution was from 37 to 52 yr. All patients had no severe complications such as liver disease, urinary disease, infectious disease, and severe anemia. All tests were performed before the patients received the treatment.

Culture medium. RPMI 1640 medium (Gibco, Grand Island, N.Y.) containing 100 units/ml Penicillin G (Meiji Seika Kaisha, Ltd., Japan), 100 μg/ml Streptomycin (Meiji), 2 mg/ml sodium bicarbonate and 5.9 mg/ml HEPES was sterilized by cellulose nitrate membrane (0.45 μ pore size, Sartorius, West Germany) filtration.

WBC. Heparinized whole blood (0.2 ml) was mixed with 3 ml of culture medium containing 20% fetal calf serum (FCS, Gibco) in a Pyrex tube. Diluted blood samples (0.2 ml) were put in each well of Microtest 2 tissue culture plate (Falcon). Twelve μg of phytohemagglutinin-P (PHA-P, Difco, Detroit), which had been found to give the optimum response of lymphocyte in our experimental conditions, were added to each well and incubated at 37°C in an atomosphere of 5% CO_2 and 95% air for 5 d. At 96 h of incubation, 0.5 μCi of [^{3}H]thymidine (specific activity 5.0 Ci/mmol, Radiochemical Centre, Amersham) was added to each well. All cultures were performed in quadruple.

ILC. Lymphocyte suspensions were prepared from heparinized blood by the Ficoll-conrey gradient method. All the cells were washed three times with minimum essential medium (MEM, Nissui Co., Ltd., Japan), and the number of lymphocytes was adjusted to 1×10^6/ml by culture medium containing 20% FCS. Aliquots (0.2 ml) of lymphocyte suspensions were put in each well of Microtest 2 plate to which 3 μg of PHA-P was added just before the start of incubation. Cells were incubated for 72 h at 37°C in 5% CO_2-95% air incubator. At 65 h of incubation, 0.2 μCi of [^{3}H] thymidine was added to each well. All cultures were

performed in quadruple.

The estimation of the effect of autologous serum. Heparinized whole blood (0.2 ml) was mixed with 3 ml of MEM and centrifuged at 200× g for 15 min. The sediment was washed with MEM twice and suspended in 3 ml of culture medium containing 20% FCS in the presence or absence of 0.2 ml of autologous serum. Aliquots (0.2 ml) of these cell suspensions were put in each well and incubated in a same way as for WBC.

Harvesting technique. Cultures were collected on glass fiber filters (Reve-Angel 934AH, U.S.A.) using an automatic multiple sample harvester (Dynatech, England). The blood cells on the filter paper were washed with isotonic saline for 10 s and with cold 5% trichloracetic acid for 10 s. The filter paper was dried and transferred to a counting vial. The radioactivity of the [^{3}H]thymidine incorporated into DNA of lymphocytes was measured with 8 ml of scintillant (5 g DPO, 0.1 g POPOP/1 ℓ toluene) in a liquid scintilation counter (Packard, Model 3330). Lymphocyte response was expressed as counts per minute (CPM) of the average of quadruple cultures under the conditions described above.

RESULTS

The mean proliferative responses with WBC were significantly lower in all clinical stages than in the control group, and were progressively depressed as the clinical stages were increased (Fig. 1). On the other hand, with ILC, there was no difference in the mean proliferative responses between the control group and patients with stage 1, nor were there differences between the clinical stages. When the data were analyzed for individual subjects, several patients, regardless of clinical stages, had proliferative response below the control value (Fig. 2). Then, by classifying the proliferative responses to three ranges (over, below and in the control value), it was examined whether the distributions of the number of cases in each range were different among the control group and each stage. By the data from ILC, there was not a significant difference in the distribution between any pair of controls and the clinical stages ($\chi^2=11.0$ DF=6, $P>0.05$). For WBC, there was a significant difference in the distribution between some pairs ($\chi^2=30.1$, DF=3, $P<0.05$), for instance, the control group and stage 1 ($\chi^2=10.0$, DF=1, $P<0.05$). In other words, with the data from ILC, a line could not be drawn between the control group and cancer patients, but it could be done with WBC.

In order to estimate the effect of serum factor on the lymphocyte

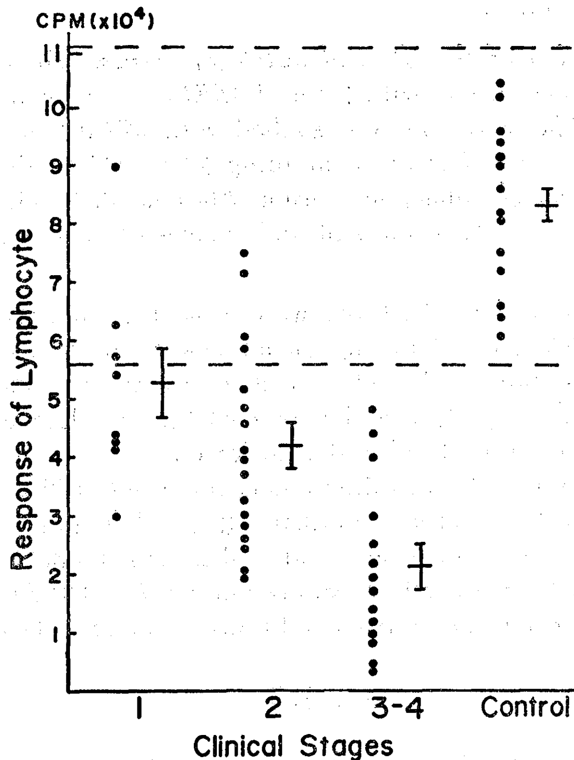

Fig. 1. Lymphocyte responses with WBC in 39 cervical cancer patients and in 14 control patients; Lymphocyte responses are expressed as CPM per culture (see Materials and Methods). Means±S.E. of each group are presented: there were significant differences between controls and stage 1 ($t=4.373$, $DF=20$, $P<0.05$), and stage 2 and stage3-4 ($t=3.554$, $DF=29$, $P<0.05$). The dotted lines enclose the control value (mean±2 S.D. of control group).

response, centrifuged blood cells were incubated with both the culture medium and the medium containing autologous serum. Fig. 3 shows the presence of autologous serum produced noticeable suppression in the lymphocyte responses in 3 of 7 patients with stage 1, 4 of 9 patients with stage 2, and 9 of 11 patients with stage 3 and 4. But in the control group, the presence of autologous serum caused no change. However, even in the absence of autologous serum, patients with early stages still showed significantly lower mean responses than the control group ($P<0.05$).

DISCUSSION

Lymphocyte responses to PHA have been studied in various kinds of

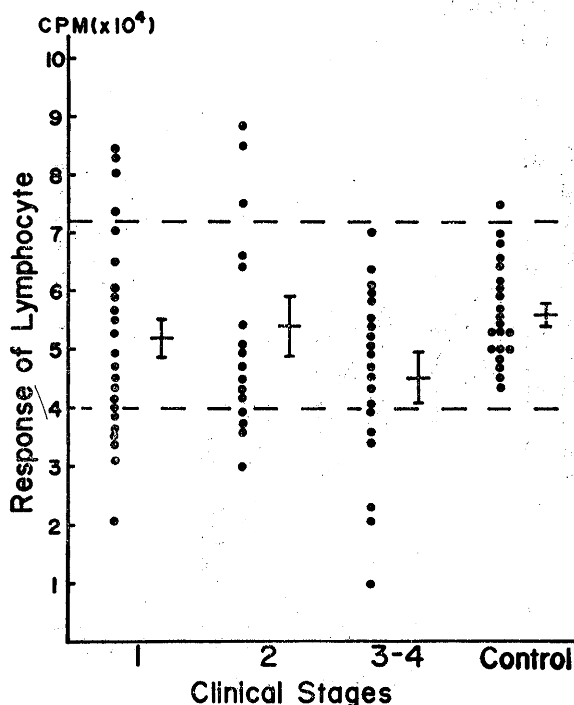

Fig. 2. Lymphocyte responses with ILC in 60 patients with cervical cancer and in 21 controls; Lymphocyte responses are expressed as CPM per culture (see Materials and Methods). Means±S.E. of each group are presented: no difference between control group and stage 1, or clinical stages each other ($P>0.05$). The dotted lines enclose the control value (mean±2 S.D. of control group).

malignancies. However, the results are still inconclusive[5-11]. Unfortunately, little information is available regarding the lymphocyte responses in cervical cancer. Hagen et al[4]. have reported the lymphocyte response in patients with cervical cancer using ILC, and noted that responses were not depressed in the non-metastatic disease, regardless of clinical stages. Similarly in the present study, by the data from ILC, cancer patients were not distinguished clearly from control group, though several patients, regardless of clinical stages, had the response below the lowest value in control group. These low responses might not be correlated with clinical stages, but exact explanation was unknown. On the other hand, with WBC,even patients with early stages showed an apparently depressed response, and the clinical stages seemed to be reflected in the response. Yamagata and Green[3] also reported the lymphocyte response with WBC in patients with cervical cancer and noted that the

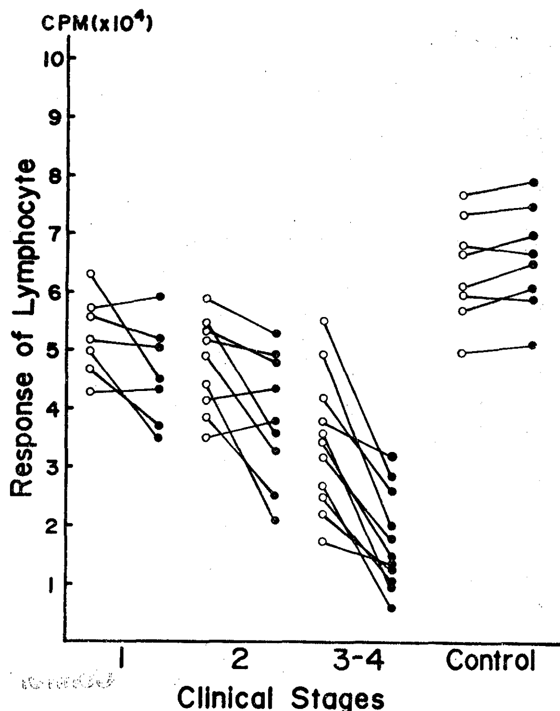

Fig. 3. Autologous serum effects on the lymphocyte responses; ○, without autologous serum. ●, with autologous serum. Lymphocyte responses are expressed as CPM per culture (see Materials and Methods).

responses were suppressed in all stages, and progressively as clinical stages increased.

It is interesting to note that WBC showed a different proliferative response from ILC in cancer patients when compared to the control group. One of the factors concerning this discrepancy might be the presence of the autologous serum in WBC. Several investigators have reported the inhibitory effects of cancer serum on the lymphocyte responses[12-17]. In the present study, it was suggested that the plasma component in WBC might play a role in reducing the lymphocyte response, though not all, in cancer patients. However, even in the absence of autologous serum, cancer patients, even early stages, had significantly lower mean response than control group. Therefore, in addition to the autologous serum, other possible explanations for the discrepancy of the two methods should be considered, such as the influence of the absolute number of lymphocytes, the constitution of lymphocytes subgroups, and the other leucocytes. Yamagata and Green[3] reported the relationship between lymphocyte counts in peripheral blood and proliferative

responses with WBC, and noted that they had tendency to correlate to each other. Unexpectedly, in our laboratory, there was no correlationship between them (unpublished data). As for constitution of lymphocytes subgroups, though this is not direct explanation, Park and Good[18] stated as follows. The current methods of ILC did not provide a quantitative and reproducible means of assesing T-cell function, since ILC resulted in random selection of lymphocytes subgroups in the peripheral blood, but these improprieties were eliminated in WBC. Concerning the influence of other leucocytes to the proliferative response, McFarland[19] said that pure lymphocytes reacted suboptimally when compared to those recombined with other leucocytes separated in the course of isolating lymphocytes. Gough et al[20]. also said that presence of polymorphs was necessary for the lymphocyte transformation. It is unknown whether the other leucocytes of cancer patients had a low abifity of enhancing the proliferative response, but this hypothesis might not be repudiated.

In conclusion, the method of whole blood culture appears to give clinically useful information concerning the immune status of cancer patients, much better than ILC. Further examinations of the factors are necessary, such as those described above, which might cause the discrepancy between the two methods in cancer patients. However, our data would indicate at least that the autologous serum in WBC might be one of those factors.

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