Correlation Between Serum Alpha-Globulin and Plasma Inhibitory Effect on PHA-Stimulated Lymphocytes in Colon Cancer Patients[1] (36189)

CLEMENT C. S. HSU[2] AND PAUL LOGERFO
(Introduced by H. J. Hansen)
Institute of Cancer Research, Francis Delafield Hospital, Columbia Presbyterian Medical Center, New York, New York 10032

It has generally been accepted that there is an immunologic surveillance mechanism mediated by cellular immunity, which recognizes and destroys the cells which have undergone neoplastic transformation (1). There is also evidence that the lymphocyte, the main participant in cellular immunity, is responsible for tumor resistance and rejection (1). Because the *in vitro* lymphocyte response to phytohemagglutinin (PHA) probably indicates the presence of immunologically responsive lymphocytes (2), the recent reports that plasma from cancer patients inhibited the lymphocyte response to PHA is of great interest (3–5). This may indicate that there is a humoral factor in cancer patients which would supress the lymphocyte activity and cause the tumor to grow without inhibition once it is established; or if it is present before the development of the tumor, it may cause the failure of the surveillance mechanism with subsequent escape of neoplastic cells and establishment of their growth.

To study the relationship of this humoral factor and the course of the disease it is important to study patients in the early stages and correlate statistically the plasma inhibitory effects with various indices of the disease. However, the lymphocyte response to PHA in different individuals and in each individual on different occasions varies. Therefore the results of inhibition of PHA-stimulated lymphocytes from different experiments cannot always be compared to each other unless a particular plasma is used as a standard and tested in all experiments, or all plasmas are tested on lymphocytes from a single donor in the same experiment. In this study, we collected plasma from 15 patients with colonic neoplasms, mostly in early stages, and 15 control subjects and tested their effects in the same experiment. We have found that there is no difference statistically between our cancer and control groups. However, within the cancer group, the inhibitory effect of the plasma is correlated with the serum level of alpha-globulin and alpha-globulin level is correlated with carcinoembryonic antigen (CEA) level.

Materials and Methods. The cancer group consisted of 15 patients who were admitted with the diagnosis of colon cancer, subsequently proven by surgery. All had adenocarcinoma except Tom who had squamous cell carcinoma of the rectum (Table I). They had not received any treatment, had not taken any drugs for at least 2 days before the plasma collection and did not have any evidence of other diseases including those which have been reported to have plasma inhibitory factors, *i.e.*, uremia, cirrhosis, tuberculosis, syphilis, allergies, Hodgkin's disease, idiopathic steatorrhea, ataxia telangiectasia, and multiple sclerosis (6). Their clinical data were collected and serum protein electrophoresis was performed on cellulose acetate (Veronal buffer, pH 8.6; and ionic strength, 0.075; Beckman Analytrol). The stage of the cancer was determined histologically and classified as follows: Stage A: those with invasion not beyond submucosa of the colon; Stage B: those with invasion not beyond muscularis layer; Stage C: those with invasion into or beyond the serosa, local lymphatic spread or involvement of adjacent organs; and Stage D: those with distant

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[2] Present address: Division of Medical Genetics, Department of Pediatrics, Mt. Sinai School of Medicine, New York, N.Y. 10029.

TABLE I. The Results of Stage, CEA, CPM (of Expt. II) and Serum Protein Electrophoresis.

Name	Stage of cancer	Serum CEA level	Mean cpm (Expt. II)	Total protein	Albumin	Alpha 1	Alpha 2	Beta	Gamma[a] 1	Gamma[a] 2
Gra	D	100	3034	6.4	2.9	0.5	1.3	0.3	0.4	1.0
Bec	B	60	6553	7.0	3.4	0.3	1.1	1.0	0.2	1.0
Wur	A	10	9578	6.0	3.0	0.3	0.8	0.6	0.4	0.9
Amb	B	0	4371	6.3	3.3	0.4	0.9	0.8	0.4	0.7
Tuc	D	0	8801	6.3	3.5	0.3	0.7	0.8	0.3	0.7
Chw	B	0	3671	5.4	2.5	0.2	0.6	1.0	0.3	0.8
Tom	Sq. Ca.[b]	50	2401	7.8	4.1	0.4	1.2	0.7	0.4	1.0
Thi	C	40	8159	7.0	3.7	0.3	1.1	0.9	0.3	0.8
War	B	0	7906	6.8	3.7	0.3	0.9	1.0	0.2	0.7
Jen	A	0	7309	6.9	3.5	0.2	0.8	0.7	0.8	0.9
Mon	A	0	10343	7.0	3.8	0.2	0.8	0.8	0.1	1.2
Sea	B	24	4869	6.4	3.7	0.2	0.9	0.7	0.3	0.6
Scc	D	100	5155	6.3	3.0	0.4	1.0	0.9	0.2	0.9
Tet	B	100	1259	6.7	2.4	0.4	1.2	0.5	1.2	1.0
Oet	B	8	6249	6.6	3.2	0.3	0.9	0.8	0.6	0.8

[a] Normal values (g/100 ml): albumin: 3.5–4.8; alpha-1: 0.2–0.4; alpha-2: 0.5–0.9; beta: 0.7–1.2; gamma-1: 0–0.3; gamma-2: 0.9–1.5.

[b] Squamous cell carcinoma.

metastases. Our control group consisted of 15 healthy personnel working in the laboratory.

The plasma was collected with a B-D disposable plastic syringe containing 10 units of Pan-heparin (Abbott)/1 ml of blood. The plasma devoid of detectable cellular elements was separated by centrifuation at 1000*g* for 10 min. Then it was frozen at −20° until use.

Lymphocyte cultures were set up as reported previously (6). Each culture tube contained 0.5 × 10^6 lymphocytes in 0.5 ml of medium consisted of medium 199 with 20% plasma, 200 units/ml of penicillin, and 200 μg/ml of streptomycin. Triplicate tubes were used for each plasma. PHA-M (Difco, lot No. 530281) was added to each tube at the same dose (0.0025 ml of reconstituted volume). The cultures were maintained in water saturated 5% CO_2–95% air at 37° for 3 days. Twenty-four hours before harvesting the cells, tritiated thymidine (NEN, sp act 1.9 Ci/mole), 0.5 μCi each, was added to the culture. An aliquot of culture was taken at the time of harvest and cells were examined for viability with trypan blue stain. The viability was from 90 to 95%; and no difference was noted between all the plasmas.

After harvesting, cells were washed twice with phosphate buffer in saline (pH 7.2) and twice with 5% trichloroacetic acid. The precipitates were then dissolved in 0.5 ml of Hyamine solution. Ten milliliter of Bray's solution was added and radioactivity was counted in a Beckman Scintillation counter. The mean cpm of triplicate cultures was used for the statistical calculation. The precision of the experiments as expressed by mean percent maximum variation among triplicates ± SD is 22.7 ± 14.8%, where

$$\text{percent maximum variation} = \frac{\text{maximum variation (cpm) among triplicates}}{\text{mean (cpm) of triplicates}} \times 100\%.$$

The serum CEA level was measured using a radioimmunoassay with goat anti-CEA antiserum and Zirconyl phosphate gel as reported previously (7) and the result is expressed in nanograms per 5 ml.

Results. The experiment was repeated 2 times (I, II, and III) using 2 lymphocyte donors. The results (cpm) of all experiments are shown in Fig. 1. Although the Expt. II showed higher cpm due to higher lymphocyte response to PHA of this donor, the relative effects of each plasma were generally the same. The mean and SD (cpm) of the cancer and the control groups in 3 experiments are also shown in the bottom of Fig. 1. On

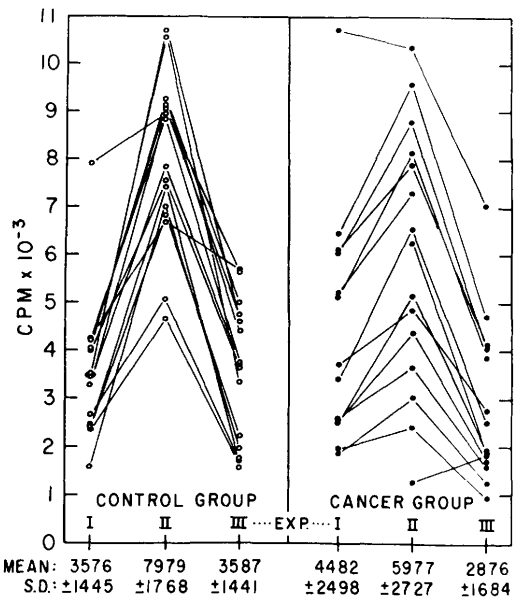

FIG. 1. The cpm of PHA-stimulated lmphocytes cultured in plasma of 15 controls and 15 cancer patients: Each point represents the mean of 3 replicates and the line connects the results of individual plasma in three experiments.

the whole there is no difference between the 2 groups. However, within the cancer group, those with higher serum CEA levels tend to have lower cpm, although a significant statistical correlation using a nonparametric test could not be obtained ($r_s = -0.43$; r_s for $n = 15$ is 0.44 for 5% level and 0.62 for 1% level of significance). Using the same test, a negative correlation between cpm and α-globulin levels (the sum of α-1 and α-2 globulins) and a positive correlation between CEA and α-globulin levels were obtained which were significant at 5% ($r_s = -0.53$) and 1% ($r_s = 0.84$) levels, respectively, within the cancer group. No correlations were noted between cpm and blood type, serum levels of blood urea nitrogen, uric acid, calcium, phosphorous, liver function tests, and the levels of other fractions of serum proteins.

Discussion. Although in certain situations, such as uremia, plasma factor seems to be responsible for the impaired cellular immunity (8), clinical significance of this factor has been questioned because of PHA precipitation by some serum proteins [see review in Ref. (6)]. In recent years, serum alpha-globulin has been shown to be immunosuppressive (9, 10), and has been postulated to be the immuno-regulatory humoral factor which suppresses the immune response and prevents lymphoid proliferation (11). In ataxia telangiectasia the presence of plasma inhibitory factor which blocks lymphocyte response to PHA and pokeweed mitogen seems to be related to the increased serum alpha-2 globulin levels (12). The recent report that kidney transplant recipients showed elevated alpha-2 globulin levels during acute rejection periods and that their midrejection serum inhibited lymphocyte response to PHA appears to support the presence of this immuno-regulatory feedback mechanism (13).

In our study, although there was no statistical difference in cpm between the control and the cancer patient as a group, cpm within the cancer group tended to be lower in plasma with higher CEA level, which had been found to be higher in more advanced stages of cancer (7). Even though this correlation did not reach statistical significance, it is supported by the fact that the serum alpha-globulin level is correlated with both plasma inhibitory effect and serum CEA levels. Since our cancer patients consisted mostly of early stages of the disease, this probably indicates that the plasma inhibitory factor is not present in large amounts in early cancer patients and increases with the advance of the cancer. The finding by others (4) that the cpm was significantly lower in more advanced groups of cancer patients seems to be consistent with our view. Our results also suggest that the suppressed lymphocyte response is related to increased alpha-globulin. Therefore, it is possible that this plasma inhibitory effect is caused by direct blocking of the lymphocyte response to PHA by some factor produced by the tumor or by increased production of alpha-globulin stimulated by CEA or some other factors from the tumor. Since the human tumor probably contains tumor-specific transplantation antigen (14–17), another possibility is that this is caused by the increase in immuno-regulatory alpha-globulin mediated through the similar mechanisms as in kidney transplant recipients during rejection.

Conclusion. The effects of plasma from 15 normal individuals and 15 early colon cancer patients on PHA-stimulated lymphocytes were studied by testing on lymphocytes from a single donor in the same experiment to permit statistical analysis. As a group there was no difference between normal individuals and cancer patients. However, the plasma inhibitory effect tended to be more pronounced in patients with higher serum carcinoembryonic antigen levels which had been found to be higher in advanced stages of cancer. This probably indicates that the plasma inhibitory factor is present in increased amount in later stages of cancer but not in the early stages. A positive correlation was also found between plasma inhibitory effect and serum α-globulin level and between serum levels of α-globulin and carcinoembryonic antigen.

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