Eur J Cancer Clin Oncol, Vol. 17, No. 10, pp. 1105–1115, 1981.
Printed in Great Britain.

0277-5379/81/101105-10$02.00/0

Evaluation of Cellular Fluidity of Peripheral Blood Lymphocytes from Patients with Urogenital Cancers by a Fluorescence Polarization Technique: PHA Response and Prognosis

SADAAKI SAKAMOTO,* HIROSHI MAEDA,† KOZO YAMASAKI,* SHOGO AIKAWA,† and YORIO HINUMA†‡

*Departments of *Urology and †Microbiology, Kumamoto University Medical School, Honjo* 1-1-1, *Kumamoto, Japan* 860

Abstract—*Cellular fluidity of peripheral blood lymphocytes of* 28 *healthy donors,* 24 *patients with non-malignant urogenital diseases and* 61 *patients with urogenital cancers was measured after stimulation of phytohaemagglutinin (PHA) by a fluorescence polarization (P) method described by Cercek and Cercek and modified by us. P-values of lymphocytes without stimulation in each group were almost the same. The lymphocytes from healthy donors or patients with non-malignant diseases responded to PHA to a much greater extent than those from cancer patients. The stimulation index (SI), which quantifies the responsiveness of lymphocytes to PHA, was calculated based on the P-values. There was a significant difference in SI values between the control group, including healthy and non-malignant donors, and the malignant group* ($P < 0.005$). *In patients with malignant diseases, SI values were found to have close relations to the prognosis, stage, grade and lymphoid reaction. The present results indicate that the measurement of PHA response based on cellular fluidity of peripheral blood lymphocytes may be useful in diagnostic and prognostic evaluation of urogenital cancers.*

INTRODUCTION

IN MALIGNANT diseases, the immunological functions are known to be impaired, as revealed by several immunological parameters such as leukocytes migration inhibition, antibody-dependent cytotoxicity and T-cell function tests [1]. The membrane fluidity of tumor or transformed cells is known to become higher than that of the normal cells [2, 3]. In 1974, Cercek *et al.* reported the responsiveness of peripheral blood lymphocytes to stimulation by phytohaemagglutinin (PHA) or so-called cancer basic protein (CaBP). These responses were observed by cellular fluidity (or structuredness of cytoplasmic matrix, SCM) measured by fluorescence polarization (P) technique, for which fluorogenic fluorescein diacetate (FDA) was utilized as an intracellular probe [4–7]. The lymphocytes from healthy donors were stimulated by PHA and resulted in an increased fluidity. On the contrary, stimulation by CaBP resulted in no response. On the other hand, the lymphocytes from patients with malignant tumors were stimulated by CaBP but not by PHA. Cercek and Cercek [8] applied the SCM evaluation to lymphocytes from a large number of patients with various tumors and non-malignant diseases, as well as healthy subjects, and concluded a potential application of the method for diagnosis of cancer. Takaku *et al.* [9] applied the method similar to Cercek to the patients with gastric

Accepted 18 May 1981.
Requests for reprints to Hiroshi Maeda.
‡Present Address: Virus Research Institute, Kyoto University, Kyoto, Japan.

cancer and obtained a concordant result to the previous reports. However, in spite of its potential scope of clinical usefulness the application of this method has been rather limited because of the operational tediousness.

We have extended and re-evaluated the method to the urogenital cancer patients using PHA as a stimulant. In addition, the stage and grade of tumors and patients' prognosis were correlated to the measured P-values.

A fluorescence polarization instrument used in the present study is equipped with an automatic calculator for the P-value and requires a much simpler operation.

MATERIALS AND METHODS

Donors

Blood samples were taken from 28 healthy donors, 24 patients with non-malignant urogenital diseases and 61 patients with non-treated urogenital cancers. Diagnosis in most patients of cancer was done histologically. Age, sex and underlying diseases are summarized in Table 1.

Chemicals

FDA was obtained from Dojin Chemical Co., Ltd., Kumamoto, Japan. Carbonyl iron and PHA (Wellcome, purified PHA HA 16) were purchased from Wako Chemical Industries, Osaka, Japan and from Wellcome Research Laboratories, Beckenham, England respectively. Ficoll 400 and Conray 400 (sodium iothalamic acid, 66.4% w/w) were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden and from Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan respectively.

Instruments [10, 11]

Polarization spectrofluorometry was done with a JIMCO polarization spectrofluorometer Model MAC-2 type HR-1, Japan Immunoresearch Co., Ltd., Takasaki, Japan. This instrument is equipped with a primary polarizer, an excitation filter (490 nm), a cell housing, an analyzer polarizer, an emission filter for 520 nm and a photomultiplier with an amplifier system. A built-in microcomputer is also furnished in the instrument, which calculates the P-value and fluorescence intensity (FI). Each P-value in the present study is a multiple of about 2.0×10^3 of a real value.

All filters are three-cavity filters (Ditric Optics Inc., Hudson, Mass, USA) with near monochromatic performance, and with low extinction (about 50% transmission) and little depolarization.

A refrigerated water circulator Model RTE-8 (Neslab Inst. Inc., Portmouth, N.H., USA) was employed to maintain the temperature constant within ± 0.05°C.

Table 1. *Age, sex, underlying diseases and stimulation index (SI) of lymphocytes from healthy donors, patients with non-malignant diseases and cancer patients*

Materials	No. of case	Sex M	Sex F	Mean age (yr)	SI mean ± S.D.
Healthy donors	28	25	3	40 ± 12	28.7 ± 15.1
Patients with non-malignant diseases	24	16	8	50 ± 17	35.8 ± 19.3
Total	52	41	11	48 ± 16	34.6 ± 18.3
Patients with malignant diseases:					
Bladder cancer	36	27	9	64 ± 14	6.8 ± 9.1*
Renal cell cancer	9	7	2	63 ± 8	6.7 ± 10.5*
Renal pelvic and ureteral cancer	5	5	0	64 ± 7	6.9 ± 7.1*
Prostatic cancer	8	8	0	68 ± 9	10.5 ± 18.8†
Other cancers	3	3	0	50 ± 11	8.8 ± 6.3†
Total	61	50	11	63 ± 12	7.1 ± 10.4*

*†Statistically different from control: $*P < 0.005$, $\dagger P < 0.025$.

Lymphocytes preparation

Peripheral lymphocytes were prepared by a procedure described previously [12]. Briefly, 20 ml heparinized peripheral blood was mixed with 2 ml of 5% carbonyl iron suspended in PBS (0.01 M phosphate buffer, 0.15 M NaCl, pH 7.0, PBS) containing 5% arabic gum. The mixture was centrifuged at 400 *g* for 30 min on a density gradient comprised of 9% Ficoll and 33.4% Conray in a ratio of 2.4:1.0. A band of lymphocytes was collected at $\rho = 1.080$, washed twice gently with PBS by pipetting and re-suspended in PBS in a Falcon test tube (1.7 × 10 cm) at the concentration of 5×10^6 lymphocytes/ml. This method yielded more than 95% lymphocytes containing less than 5% monocytes and neutrophiles. The entire process of lymphocyte purification was completed within 2.5 hr. The cell suspension in PBS was kept in an incubator at 37°C and used within 1 hr after preparation.

Assessment for the condition of polarization assay

Assay medium. In the present assay system, PBS without Ca^{++} and Mg^{++} was employed since spontaneous and thermal hydrolyses of FDA, as judged by FI, were observed during preincubation with complete Dulbecco's PBS containing Ca^{++} and Mg^{++}.

Preparation of FDA solution. FDA (20 mg) was dissolved in 1 ml of chloroform (spectrograde), then diluted with PBS under vigorous stirring to give a concentration of 25 μM (stock solution) and stored frozen until use. The final concentration after 10-fold dilution with cell suspension in PBS was 2.5 μM in the assay. Since FDA concentrations of 0.25–20 μM yielded about the same P-values in the present system, a 2.5 μM FDA solution was used.

Lymphocytes concentration. When the cell concentration of the lymphocytes is more than 5×10^6/ml, the transmission of light becomes too low ($A_{520\,nm} = 0.3$) for accurate measurement. On the contrary, when the cell concentration is less than 10^5/ml, FI becomes too low. Therefore, a lymphocyte concentration of 5×10^5/ml was used.

Temperature of assay. Below 20°C, there is very little uptake of FDA into cells, while above 37°C, thermal and spontaneous hydrolyses of FDA interfere with the measurement. Temperatures of 27–32°C appeared to be appropriate. In the present study, the measurement was done at 27°C.

PHA concentration and incubation time with FDA. Within the range of 5–40/fold dilution of PHA (original solution; 400 μg/ml), the response of lymphocytes was nearly constant, as revealed by FI and P-values. Response of lymphocytes to PHA diluted more than 80-fold gave considerable variations. Therefore, 100 μl of 5-fold diluted PHA was added to 2 ml of the cell suspension in a Falcon tube and allowed to incubate at 37°C for 30 min with occasional gentle shakings.

Procedure of fluorescence polarization assay

Based on the above-described condition, the method was standardized as follows: 0.2 ml of FDA stock solution was added into a cuvette containing 2 ml of PBS which was preincubated at 27°C, followed by thorough mixing by inverting several times of the cuvette covered with a piece of Parafilm™. The cuvette was then placed in a thermostated spectropolarimeter. No thermal nor spontaneous hydrolyses of FDA were recognized by FI in solution without lymphocytes (less than 0.5 pmole/ml). A possible extracellular esterase activity, which might give a back-ground fluorescence, was also undetectable in the supernatant of the cell suspension. An aliquot of PHA-treated or non-treated lymphocyte suspension containing 1×10^6 cells in total was added to the cuvette followed by gentle shaking, as described above. Immediately after placing the cuvette in the holder, the measurement was started. P-values and FI were printed automatically.

Stimulation Index

In order to normalize these P-values in a more standard manner, a stimulation index (SI) was calculated as follows:

$$SI = \frac{P_{non} - P_{PHA}}{P_{non}} \times 100.$$

P_{non} and P_{PHA} represent P-values of non-treated lymphocytes and lymphocytes treated with PHA respectively. The SI value in each case was obtained by a mean of five SI values taken at 7 min intervals from 2 min after FDA addition to the cuvettes.

Histological evaluation

In bladder cancer, the clinical staging system of UICC [13] and the grading system of Broders [14] were used. Lymphoid reaction in bladder cancer was also examined histologically, according to the criteria of Sarma [15].

RESULTS

Fluorescence microscopy of FDA-treated lymphocytes

When lymphocytes were incubated with 2.5 μM FDA, the fluorochrome entered into the cells immediately (within 1 min) and in-

tracellular fluorescence was clearly visible under fluorescence microscopy. As shown in Fig. 1, fluorescence could be seen not only in the cytoplasm but also in the nucleus and the microsome. Fluorescence was not usually detected in the membrane. Free fluorescein added to the cell suspension was not incorporated into the cells.

PHA response of lymphocytes

Typical examples of the time courses of P-value and FI in cells from a healthy man and a patient with cancer are shown in Fig. 2(A and B respectively). P-values increased promptly and reached a maximum value about 10 min after incubation with FDA, followed by a gradual decrease with time of incubation for 90 min. FI increased gradually and reached a plateau after 60 min incubation. Lymphocytes from the healthy donor responded to PHA and exhibited an increased intracellular fluidity, as represented by a decrease of the P-value. The P-values of cells treated with PHA were about two-thirds of those of non-treated cells. This reduction of P-values or increased cellular fluidity was observed throughout the time course of measurement.

On the contrary, the levels of FI in both PHA-treated and non-treated cells were almost the same (Fig. 2A), indicating that the amount of fluorescent probe in these cells was not affected by the treatment with PHA. In the case of cancer, there was only a small difference in P-values between PHA-treated and non-treated cells (Fig. 2B). This indicated that lymphocytes from the cancer patient responded only slightly to PHA. FI values of both PHA-treated and non-treated lymphocytes from the cancer patient and those from the healthy donor were all similar (Fig. 2B).

Stimulation index and incubation time in malignant and non-malignant diseases

As shown in Fig. 3, the SI's of ten representative cases from the cancer patients and the non-malignant controls remained fairly constant throughout the whole period of measurement. The mean SI value of lymphocytes from 28 healthy donors was 28.7 ± 15.1 and that from 24 non-malignant patients was 35.8 ± 19.3. No significant difference was detected between these two groups (Fig. 4, $P > 0.5$). Therefore, these two groups could be referred to as the non-cancer control. Most cases of the control groups showed SI values higher than 17.0. Hence, an SI value of 17 can be regarded as an arbitrary critical value. When this value, 17, was applied to the controls (healthy and non-malignant), most of them fell within one S.D. unit. Only 4 cases (14%) of 28 healthy donors with no clinical evidence of malignant status showed lower values than 17.0. Three of these exceptional cases exhibited an SI of less than 7.4 in the non-malignant group. One of them was 55-year-old female with an SI of 4.7 and the other was 53-year-old male with an SI of 6.0. Both were suspected, however, to have urinary cancer because +4 urinary fribrinogen depolymelized products [16] and intermittent hematuria were observed. The latter showed class V in urine cytology. Although clinical evaluation, including open surgery, did not reveal the malignant status, they might be reflecting a pre-malignant rather than a non-malignant state. A third patient was a 32-year-old male with an SI of 7.4. Laboratory examinations showed no evidence of malignant status and post-operative histological examination also failed to confirm any malignant indication. The reason for the low SIs in three cases remained unclear.

The mean SI value of lymphocytes from 61 patients with urogenital cancers was 7.1 ± 10.4, which was significantly different from the control groups ($P < 0.005$) (Fig. 4). In 13 cases (21.3%), the SI value was below 0, with the lowest being -22.9. This cancer group includes the following five different urogenital cancers (no. of cases): bladder cancer (36); renal cell cancer (9); renal pelvic-ureteral cancer (5); prostatic cancer (8); and 3 other cancers: two testicular cancers and one penile cancer. Their SI values are shown in Table 1 and Fig. 5. There was no significant difference among these subgroups. There were four exceptional patients (6.5%) showing SI values of more than 17: two prostatic cancers of stage I, one renal cell cancer of stage IV and one bladder cancer of stage Tl.

Relation between SI and prognosis and malignancy of cancer

As shown in Table 2, SI values and the prognosis of patients with cancer were closely correlated. Group A showed a statistically significant difference from group B-C. However, there was little difference between group B and group C.

SI values for each stage of bladder cancer are shown in Fig. 6. Mean SI values were: 13.7 ± 4.1 in stage T1; 9.4 ± 3.7 in T2; 2.6 ± 3.2 in T3; and -5.0 ± 8.3 in T4. Each SI value was significantly lower than that of control group ($P < 0.005$) and there were significant differences in mean SI values between T1 and T2 ($P < 0.05$), T2 and T3 ($P < 0.01$), T1 and

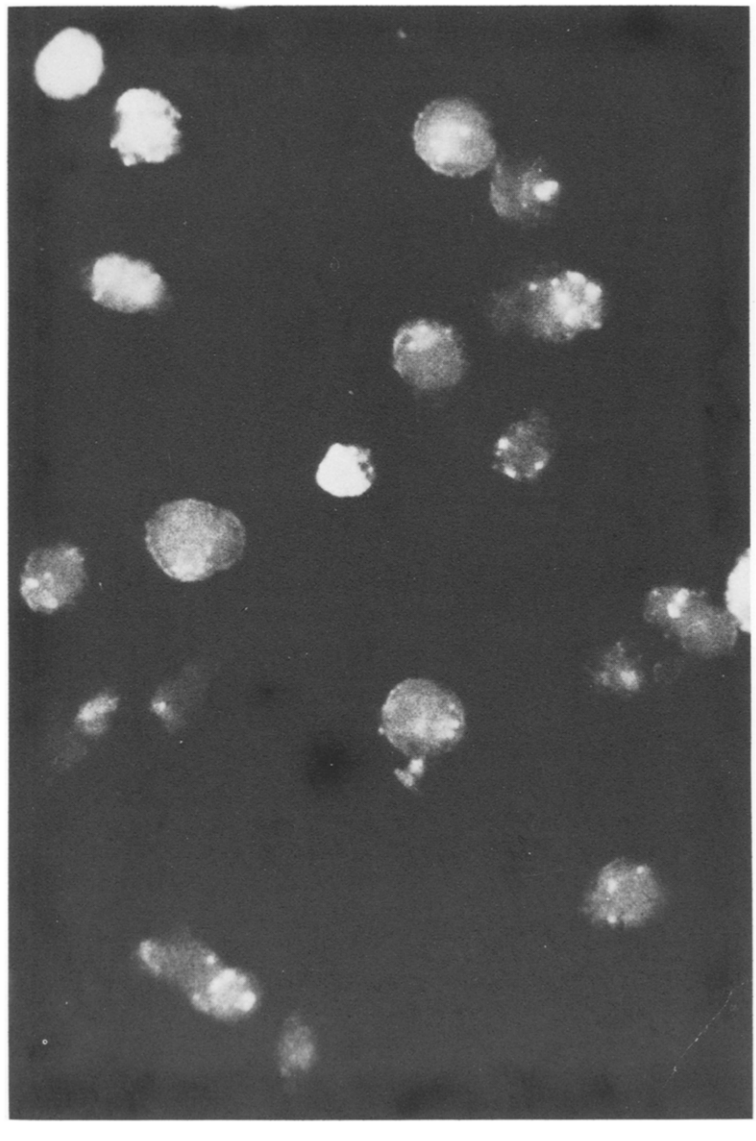

Fig. 1. Fluorescent microphotogram of lymphocytes one minute after treatment with FDA. Fluorescence could be seen not only in the cytoplasm but also in the nucleus and microsomes (×600).

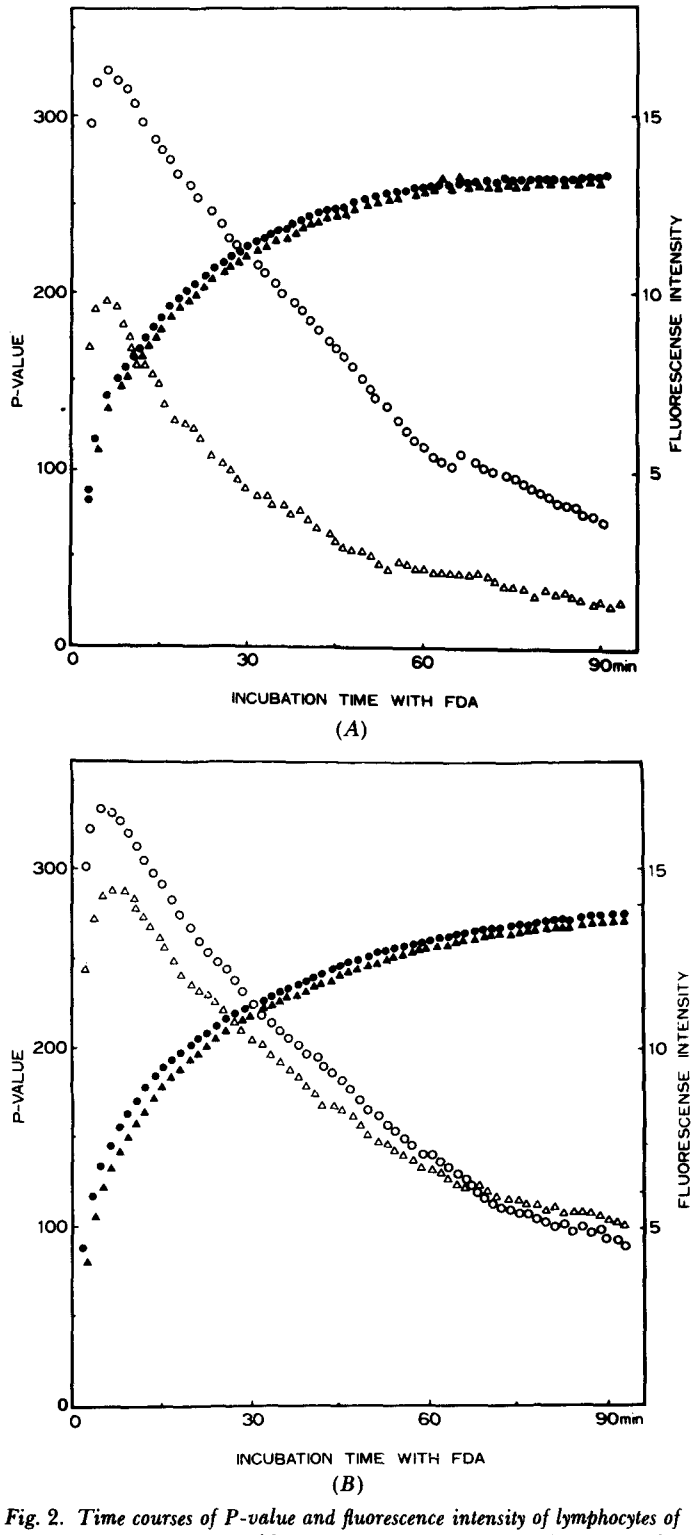

Fig. 2. Time courses of P-value and fluorescence intensity of lymphocytes of representative cases. (A) healthy donor; (B) cancer patient. Open and solid marks show P-values and FI respectively, and circles and triangles indicate PHA non-treated and treated lymphocytes respectively.

T4, T2 and T4, and T3 and T4 ($P < 0.005$) respectively.

SI values for each grade of bladder cancer are shown in Fig. 7. Mean SI values were: 14.7 ± 3.8 in grade I, 11.0 ± 4.2 in grade II and -0.7 ± 8.4 in grade III. Each value was significantly lower than that of the control group ($P < 0.005$). There were significant differences between grade I and III and between grade II and III ($P < 0.005$). No significant difference between grade I and II was recognized.

In the patients with prostatic diseases, SI of cancer group ($n = 8$) was significantly lower

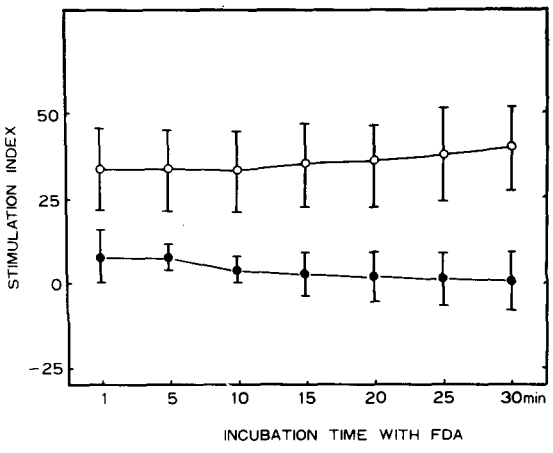

Fig. 3. Representiative time course of stimulation indices (SI). Figure shows mean SI values in every 5 minutes of incubation times in cancers (●) and non-cancer control (○); 10 patients each.

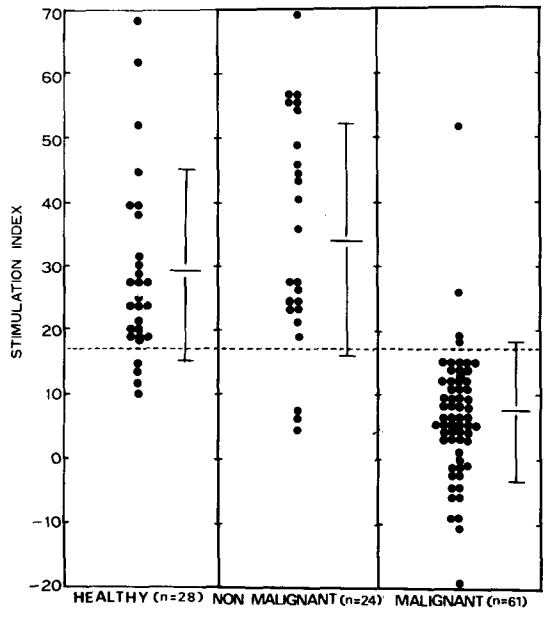

Fig. 4. Stimulation indices of healthy donors, patients with non-malignant diseases and cancer patients. Vertical bars: mean ± S.D. A dashed line shows an arbitrary critical value (SI: 17.0).

(10.5 ± 18.8) than that of benign prostatic hypertrophy (59.4 ± 18.2, $n = 7$) ($P < 0.05$).

SI and lymphoid reaction in bladder cancer

According to the histological criteria of Sarma, lymphoid reaction in bladder cancer was divided into four groups: negative (−); slight (+); moderate (+ +); and marked (+ + +). The results are summarized in Fig. 8. The mean SI values for the four groups of lymphoid reaction were: (−), 14.2 ± 3.5 ($n = 9$); (+), 13.4 ± 3.3 ($n = 5$); (+ +), 1.4 ± 7.0 ($n = 8$); and (+ + +), 0.21 ± 10.8 ($n = 7$). There were no significant differences in SI values between (−) and (+) and between (+ +) and (+ + +) respectively. However, the combined mean value

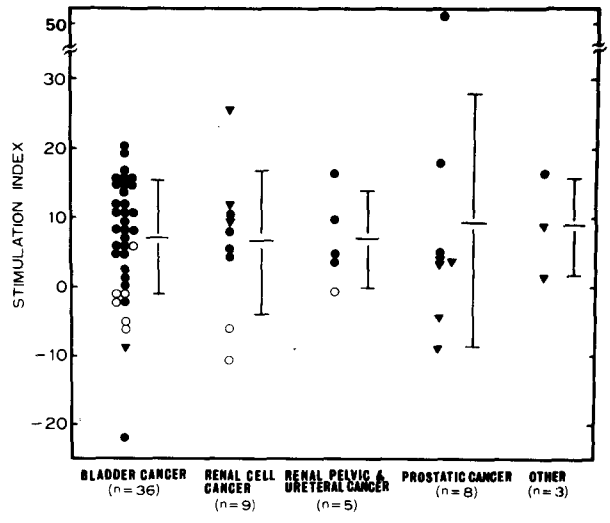

Fig. 5. Stimulation indices of various cancers. Vertical bars: mean ± S.D. Open circles, closed circles and closed triangles show SI of patients who died within one year after this test, that of patients without metastasis who are still alive and that of patients who are still alive but with metastases respectively.

of both (−) and (+) was significantly different from that of both (+ +) and (+ + +) combined ($P < 0.025$). The results indicate some relation between SI value and grade of lymphoid reaction in bladder cancer.

DISCUSSION

Cercek and Cercek [5, 8, 17] emphasized that the success of the test depends on fastidious attention to procedural details such as preparation of lymphocytes, use of Dulbecco's PBS containing Ca^{++} and Mg^{++} at pH 7.4, meticulous control of temperature, preparation of FDA solution and wave length selection. In practice, the tediousness resides primarily in such operations as the manual filtration step to remove the free fluorescein which leaked out of the lymphocytes, fixing of the polarizer by rotating 90° each time and opening and closing

Table 2. Stimulation index (SI) in relation to the prognosis of patients with cancer

Group*	No. of cases	SI mean ± S.D.
A	9	−3.0 ± 4.6†‡
B	11	4.5 ± 9.8†
C	41	10.4 ± 10.0‡

*(A)Patients who died within one year after the test; (B) Patients who are still alive with distant metastases; (C) Patients who are still alive without metastasis.
†‡Statistical evaluation. †$P < 0.05$, ‡$P < 0.005$.

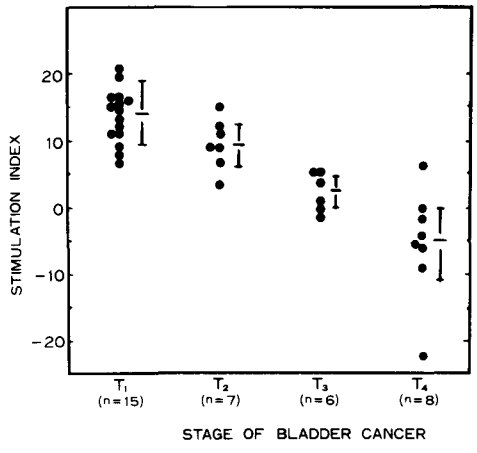

Fig. 6. Stimulation indices in reference to the clinical stage of bladder cancer. Vertical bars: mean ± S.D.

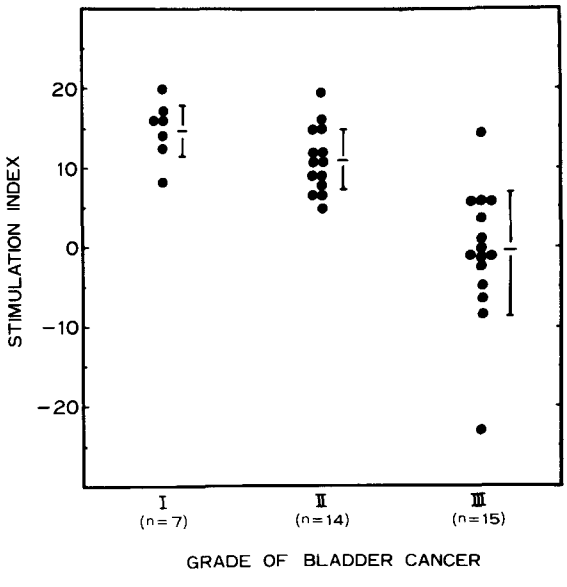

Fig. 7. Stimulation indices and grade of bladder cancer. Vertical bars: mean ± S.D.

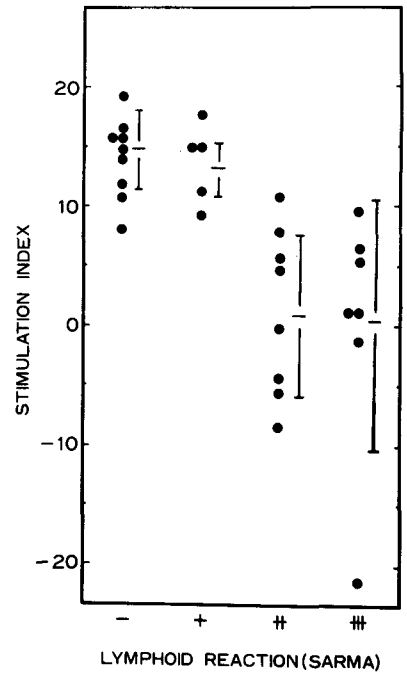

Fig. 8. Stimulation indices and lymphoid reaction in bladder cancer. Vertical bars: mean ± S.D.

of the lid for each point of measurement. Furthermore, equipment requires a correction for grating for the depolarization effect of the optical system for the calculation of P-values. These drawbacks may be the reasons for the limited application [9, 18]. Meantime, a controversial result was reported for the SCM test [19]. Therefore, operational refinements in the instrument are nessessary to overcome these drawbacks. In this regard our instrument has been improved in some of these points as described.

We have re-evaluated the experimental conditions, including the gradient centrifugation, concentrations of both FDA and PHA, effect of pH of cell suspension, treatment time of PHA and concentration of extracellular fluorescein. The results obtained were essentially similar to that of Cercek [8]. However, FDA, diluted with Dulbecco's PBS, pH 7.4, was gradually hydrolysed, perhaps due to the slight alkalinity as judged by FI. Therefore, free fluorescein, which Cercek described as effluent fluorescein from lymphocytes [8, 20], may contain these hydrolysed free fluorescein outside of cells. In our assay condition with a detection limit of about 0.5 pmol/ml, the extracellular fluorescein was undetectable up to 30 min. In addition, we have observed that some commercial PHA was contaminated with esterase which interfered with the measurement.

A previous report by Cercek [8] on lymphocytes from 270 healthy donors and 30 nonmalignant diseases showed that 98% of them responded to PHA stimulation and only 2% responded to CaBP. Of 272 cancer patients, 93.4% responded to CaBP but none of them responded to PHA. Another 5.5% of the cases responded to both CaBP and PHA at the same time. Thus, 269 out of 272 (98.9%) could be diagnosed as cancer by response to CaBP by this method. There were only 3 false negative cases (1.1%).

We undertook the present study initially with PHA and CaBP, but our preliminary results with CaBP were rather inconsistent. A part of the reason may be a lack of identity with CaBP of Cercek *et al.* in its biochemical characterization [7]. Therefore, we used commercial purified PHA only as the lymphocyte stimulant, of which the chemical characteristics are

established. In the present results, most of non-malignant cases responded to PHA (SI: 34.6 ± 18.3; Table 1). Only 4 of 28 healthy donors and 3 of 24 patients with non-malignant diseases showed SIs of less than 17.0 (Fig. 4). In spite of these minor discrepancies, the mean SI values between controls and cancer exhibited a statistically significant difference ($P < 0.005$).

It is anticipated that immunologically competent patients have a better prognosis than those with less competent patients. To clarify this, a number of investigations for the assessments of immunological responsiveness of urogenital cancer patients have been reported, such as cell mediated cytotoxicity [22], skin test [23, 24], *in vitro* lymphocyte blastogenesis [24] and mixed cell agglutination using human antisera [25]. In addition, a relationship between the grade and stage of the tumor and immunocompetence have been described [23]. In the present study a significant difference between cancers and controls in the response of lymphocytes to PHA is demonstrated, although a few exceptional cases can be seen (Fig. 4). The present results also suggested that SI exhibited a close correlation with both tumor stages and grades of bladder cancer. Furthermore, it was very interesting that SI values were closely related to prognosis of cancer patients, as shown in Table 2. The results also showed some correlation of SI to the lymphoid reaction (lymphatic infiltration) in tumor tissue. The present results, therefore, suggest that the measurement of SI may be useful in diagnosis and estimation of prognosis of urogenital cancers.

One may be able to explain this suppressed state of lymphocytes to the PHA stimulation by the effect of the humoral immunosuppressive components in cancer patients and/or the lymphocytes becoming unresponsive by such factors at cellular level [26]. Alternatively, the results may also indicate that lymphocytes that had infiltrated into the cancer tissue recognized the tumor specific antigen [27] from cancer patients, which led to the response to CaBP as revealed by the alteration in the cellular fluidity [4, 8]. One can argue, however, that such altered PHA responses in the cancer patients may be a result of change in the lymphocyte subclass population. This possibility seems unlikely, though, since altered population of subclass was not generally observed [28].

Acknowledgements—The authors would like to thank Drs. K. Ikegami and J. Ogata of the Department of Urology, Kumamoto University Medical School for their discussion, interest and encouragement.

REFERENCES

1. HARRIS JE. Immune deficiency states associated with human malignant disease. In: HARRIS JE, SHINKOVICS JG, eds. *The Immunology of Malignant Disease.* St. Louis: C. V. Mosby, 1976: pp. 283–369.
2. SHINITZKY M, INBER M. Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. *J Mol Biol* 1974; **85**: 603–615.
3. INBER M. Fluidity of membrane lipids: A single cell analysis of mouse normal lymphocytes and malignant lymphoma cells. *FEBS Lett* 1976; **67**: 180–185.
4. CERCEK L, CERCEK B, FRANKLIN CIV. Biophysical differentiation between lymphocytes from healthy donors, patients with malignant diseases and other disorders. *Br J Cancer* 1974; **29**: 345–352.
5. CERCEK L, CERCEK B. Involvement of cyclic-AMP in changes of the structuredness of cytoplasmic matrix (SCM). *Radiat Environ Biophys* 1974; **11**: 209–212.
6. CERCEK L, CERCEK B. Changes in the structuredness of cytoplasmic matrix of lymphocytes as a diagnostic and prognostic test for cancer. In: BUCALOSSI P, VERONESI V, CASCINELLI N, eds. *Proceedings of the XIth International Cancer Congress, Florence., Cell Biology and Tumor Immunology.* Amsterdam: Excerpta Medica, 1975: Vol. 1, pp. 318–323.
7. CERCEK L, CERCEK B. Changes in the structuredness of cytoplasmic matrix (SCM) in human lymphocytes induced by PHA and cancer basic protein as measured in single cells. *Br J Cancer* 1976; **33**: 539–543.
8. CERCEK L, CERCEK B. Application of the phenomenon of changes in the structuredness of cytoplasmic matrix (SCM) in the diagnosis of malignant disorders: A review. *Eur J Cancer* 1977; **13**: 903–915.
9. TAKAKU F, YAMANAKA T, HASHIMOTO Y. Usefulness of the SCM test in the diagnosis of gastric cancer. *Br J Cancer* 1977; **36**: 810–813.
10. MAEDA H. Assay of an antitumor protein, neocarzinostatin and its antibody by fluorescence polarization. *Clin Chem* 1978; **24**: 2139–2144.

11. MAEDA H, NAKAYMA M, IWAOKA D, SATO T. Assay of angiotensin I by fluorescence polarization method. In: FUJII S, MORIYA H, SUZUKI T, eds. *KININ-II: Biochemistry, Pathophysiology, and Clinical Aspects.* New York: Plenum Press, 1979: pp. 203–211.
12. KATSUKI T, HINUMA Y, YAMAMOTO N, ABO T, KUMAGAI K. Identification of the target cells in human B lymphocytes for transformation by Epstein–Barr virus. *Virology* 1977; **83**: 287–294.
13. UICC (INTERNATIONAL UNION AGAINST CANCER). Malignant tumor of the urinary bladder. In: HARMER MH, ed. *TNM-Classification of Malignant Tumor*, 3rd Edn., Japanese. Geneva: International Union Against Cancer 1978: pp. 113–117.
14. BRODERS AC. Epithelium of genitourinary organs. *Ann Surg* 1922; **75**: 574–578.
15. SARMA KP. The role of lymphoid reaction in bladder cancer *J Urol* 1970; **104**: 843–849.
16. MARTINEZ-PINEIRO JA, PERTUSA C, MAGANTO E, ZANCAJO VG, MAGALLON M, LOSADA G, ORGEGA F. The urinary fibrinogen degradation product (FDP) in bladder cancer. *Eur Urol* 1978; **4**: 348–350.
17. CERCEK L, CERCEK B. Effects of osmolarity, calcium and magnesium ions on the structuredness of cytoplasmic matrix. *Radiat Environ Biophys* 1976; **13**: 9–12.
18. PRITCHARD JAV, SUTHERLAND WA. Lymphocyte response to antigen stimulation as measured by fluorescence polarization (SCM). *Br J Cancer* 1978; **38**: 339–343.
19. BAGSHAWE KD. Workshop on macrophage electrophoretic mobility (MEM) and structuredness of cytoplasmic matrix (SCM) test. *Br J Cancer* 1977; **35**: 701–704.
20. CERCEK L, CERCEK B, OCKEY CH. Structuredness of the cytoplasmic matrix and Michaelis–Menten constants for the hydrolysis of FDA during the cell cycle in Chinese hamster ovary cells. *Biophysik* 1973; **10**: 187–194.
21. PRITCHARD JAV, SUTHERLAND WH, TEASDALE C, WHITEHEAD RH, DEELEY TJ, HUGHES LE. The macrophage electrophoretic mobility (MEM) test. An investigation of its value as a routine laboratory test in the detection of malignant disease. *Ann Clin Res* 1978; **10**: 71–74.
22. O'TOOLE C, UNSGAARD B, ALMGARD LE, JOHANSSON B. The cellular immune response to carcinoma of the urinary bladder: Correlation to clinical stage and treatment. *Br J Cancer* 1973; **28** (suppl. 1): 266–275.
23. CATALONA WJ, SHRETIEN PB. Correlation among host immunocompetence and tumor stage, tumor grade and vascular permeation in transitional cell carcinoma. *J Urol* 1973; **110**: 526–528.
24. MERRIN C, HAN T. Immune response in bladder cancer. *J Urol* 1974; **111**: 170–176.
25. DECENZO JM, HOWARD P, IRISH CE. Antigen detection and prognosis of patients with stage A transitional cell bladder carcinoma. *J Urol* 1975; **114**: 874–878.
26. KAMO I, FRIEDMAN H. Immunosuppression and the role of suppressive factors in cancer. *Adv Cancer Res* 1977; **25**: 271–313.
27. HELLSTORÖM KE, BROWN JP. Tumor antigen. In: SELA M, ed. *The Antigen.* New York: Academic Press, 1979: Vol. 5, pp. 1–112.
28. CERCEK L, CERCEK B. Effects of osmolarity and density of gradients on the isolation of SCM-responding lymphocytes. *Br J Cancer* 1978; **38**: 163–165.