Thymus-Derived Lymphocytes in Patients With Bilharzial Urinary Bladder Cancer: Brief Communication

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ABSTRACT — The purpose of the present study was to determine the levels of peripheral blood thymus-derived (T) lymphocytes in a series of 43 Egyptian patients with bilharzial bladder cancer and 15 with chronic bilharziasis and to compare these to normal Egyptian controls. Both active and total T-cell rosettes were significantly reduced in patients with stages III and IV bladder cancer, whereas only total T-cell rosettes were reduced in patients with stages I and II disease when compared to normal controls. Patients with chronic bilharziasis had intermediate values between those with cancer and normal controls, but this difference was significant only for total T-cell rosettes. Only those patients with advanced disease (stages III and IV) had significantly lower percentages of active T-lymphocytes when compared to patients with bilharziasis. Since total T-cell levels were reduced significantly in cancer patients with both early (stages I and II) and advanced (stages III and IV) disease when compared to normal controls and patients with bilharziasis, only the number of active T-cells could be correlated with the clinical stage of disease. — J Natl Cancer Inst 59: 355-357, 1977.

Recent studies indicate that patients with bladder cancer may have decreased immunocompetence as measured by impaired delayed cutaneous hypersensitivity to skin test agents such as dinitrochlorobenzene (1-3) and tuberculin (4), reduced responsiveness of peripheral blood lymphocytes to phytohemagglutinin (2, 3), and decreased levels of thymus-derived (T) lymphocytes in their peripheral blood (4, 5). The relationship between chronic infection with Schistosoma haematobium and carcinoma of the urinary bladder has long been recognized (6). Only more recently has it been appreciated that chronic schistosomiasis also may be associated with impairment of immunologic function as manifested by decreased responsiveness of lymphocytes to both phytomitogens (7) and specific antigens (8). Conversely, there may be increased humoral antibody production directed against parasite-associated antigens, the formation of immune complexes, and the development of a multiplicity of immunopathologic lesions (9). Patients with bilharzial bladder cancer, therefore, may be doubly at risk to develop severe impairment of immunologic function, since cancer and chronic infection are both independently associated with varying levels of immunoincompetence. The purpose of the present study was to investigate one specific parameter that is associated with immune function, circulating T-cell levels, and to attempt to relate these to the clinical stage of the patient’s cancer.

MATERIALS AND METHODS

Patients. — Forty-three patients with urinary bladder cancer, 35 males and 8 females with a mean age of 42.7 years (range, 26-65 yr), were included in the present study. They had no previous treatment for their tumors, and all were clinically staged according to the Wallace classification (10). Twenty-nine patients were grouped in stages I and II, 12 in stages III and IV, and 2 could not be staged. All patients had a positive history of urinary bilharziasis, and none had been given any type of antibilharzial treatment for at least 1 month prior to this study. None of the patients had received blood transfusions or anti-inflammatory or immunosuppressive drugs. The diagnosis of carcinoma of the bladder was confirmed by histologic examination after either curative or palliative surgery.

Another group of 15 patients with S. haematobium infection, 13 males and 2 females with a mean age of 41.5 years (range, 28-53 yr), also were studied. Urine specimens from all of these patients were positive for S. haematobium ova. None of the patients had hepatosplenomegaly or ascites, and their stools were free from S. mansoni ova. Cystoscopy was performed in each patient to explore the bladder wall for any bilharzial lesions, and a biopsy specimen was obtained in each case for histopathologic examination in order to exclude malignancy. None of the patients had received antibilharzial treatment for at least 1 month prior to this investigation.

A third group of 21 individuals with no significant history of disease served as normal controls. They included 18 males and 3 females, with a mean age of 33.5 years (range, 23-50 yr).

E-rosette assay. — Lymphocytes were separated by Ficoll-Hypaque density gradient centrifugation according to the method of Bøyum (11). Lymphocyte concentration was adjusted to 4 x 10⁶ cells/ml in Hanks’ balanced salt solution supplemented with 15% fetal bovine serum. Two types of T-cell populations were examined by means of the spontaneous E-rosette assay system (12), modified from that previously described by Yu et al. (13).

The “active” T-cell population (Eₐ) was enumerated by mixing 0.2 ml of the lymphocyte suspension with an equal volume of a 0.5% suspension of washed SRBC, incubating the mixture at 37°C for 1 hour, and then centrifuging at 200 g for 5 minutes. The cell pellet was gently resuspended, and one drop of a 1% toluidine blue solution was added. The suspension was transferred to a hemacytometer grid to count the number of SRBC within 4000 lymphocytes and the number of SRBC forming cells.

ABBREVIATIONS USED: E or SRBC = sheep red blood cell(s); RFC = rosette-forming cells.

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for enumeration of RFC. A rosette was defined as a lymphocyte with three or more adherent SRBC. The percentage of RFC was evaluated microscopically by counting at least 200 cells/sample.

The total T-cell population (E2) was enumerated after incubation of lymphocytes with SRBC at 37 °C for 15 minutes, centrifugation at 200 \( \times \)g, and a final incubation at 4 °C overnight. RFC were counted as previously described.

**Statistical analysis.** — Student's t-test was used for comparison of means. The threshold of significance was fixed at the 5% level. Whenever a result was found significant, the degree of significance (P value) was given.

### RESULTS

#### Active T-Cell Population

The percentage of active T-cell rosettes in the 3 groups studied is presented in text-figure 1A. Normal controls had a value of 27.2±1.8% (mean±sd) with a range of 19.1–35.6%. Urinary bladder cancer patients had a mean of 20.0±1.6%, which was significantly lower than that obtained with controls (5<0.05). Bilharzial patients had a mean of 23.1±3.1%, which was intermediate between the normal controls and cancer patients but was not significantly different from either.

The percentage of active T-cell rosettes in patients with different stages of disease is summarized in text-figure 1B. Patients with stages I and II disease had a mean of 24.0±1.5%, which was not significantly lower than that of the normal controls. Patients with stages III and IV disease had a mean of 12.3±2.8%, which was significantly lower (5<0.001) than that of either the normal controls or patients with stages I and II disease. Patients with stages III and IV disease had significantly lower values than those with chronic bilharziasis alone, whereas no such difference was observed in patients with stages I and II disease (text-fig. 1C).

#### Total T-Cell Population

The percentages of total T-cell rosettes in the 3 groups are summarized in text-figure 1D. Controls had a mean of 63.8±1.8% (range, 55.6–72.0%), whereas bladder cancer patients had a mean of 42.5±1.7% (5<0.001 when compared to controls). Bilharzial patients had a mean of 52.0±2.4%, which was significantly lower than that of normal controls (5<0.001) and significantly higher than values for patients with urinary bladder cancer (5<0.01). The 29 patients with stages I and II disease had a mean of 45.3±1.9% compared to 36.8±3.7% for the 12 patients with stages III and IV disease (text-fig. 1E). These differences were significant (5<0.05) when compared to one another and even more so when compared to normal controls (5<0.001) or patients with bilharziasis (5<0.05 for stages I and II and 5<0.001 for stages III and IV; text-fig. 1F).

### DISCUSSION

In the present study we have observed that both active (E1) and total (E2) T-cell rosettes were significantly reduced in patients with stages III and IV bladder cancer, whereas only total T-cell rosettes were reduced in patients with stages I and II disease when compared to normal controls. Patients with chronic bilharziasis had intermediate values between those with cancer and normal controls, but this difference was significant only for total T-cell rosettes. Only those patients with advanced disease (stages III and IV) had significantly lower percentages of active T-cell rosettes when compared to patients with bilharziasis. Since total T-cell levels were significantly reduced in cancer patients with both early (stages I and II) and advanced (stages III and IV) disease when compared to both normal controls and patients with bilharziasis, only active T-cell rosettes could be correlated with the stage of disease.
Our findings may have some clinical importance, since in immune-deficiency diseases the level of active T-cell rosettes also correlated with the clinical status of disease, while total T-cell rosettes did not (14, 15). It has been suggested that active T-cells may represent a further stage of differentiation of thymus-derived lymphocytes (14) and that they may have a higher net binding affinity with SRBC than do total T-lymphocytes (14). It also has been observed that active but not total T-cells may be increased either after immunotherapy or following the in vitro incubation of lymphocytes with thymosin (16).

Several other investigators (4, 17, 18) have reported that patients with bladder cancer have reductions in the total number of T-lymphocytes, although no data were provided about active RFC. In our series of 45 patients, 25 (56%) had bladder cancer of the squamous cell type, and without exception they all had S. haematobium infection (chronic bilharziasis). In contrast, patients in the previously reported studies had mainly transitional cell carcinomas that were nonbilharzial in origin. Our present data stand in contrast to those that we reported earlier (19). In that preliminary report no significant differences in the numbers of active T-lymphocytes were detected in bladder cancer patients when compared to normal controls, but in retrospect this probably was due to the small number of patients who were studied.

Chronic S. haematobium infection is accompanied by a reduction in the total number of T-lymphocytes, total T-cell rosettes, and a higher incidence of anergy to microbial skin test antigens (El-Asfahani AM: Unpublished data), both of which are indicative of impaired cellular immunity. This is associated with a further reduction in cellular immunity following the development of bladder cancer, and it is tempting to speculate that there may be a causal relationship between the two. Further studies, however, will be required to establish this as fact.

REFERENCES