Urol Res (1987) 15:181–185

Urological Research

Lymphocyte Subsets in Urologic Cancer Patients

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Accepted: July 7, 1986

Summary. Peripheral blood T lymphocyte subsets were quantitated by monoclonal antibodies in 91 patients with urologic malignancies as well as eight age-matched controls. In general, the presence of cancer and increasing stage were associated with a relative decrease in helper and an increase in suppressor T-cell activity as well as a decreased T4/T8 cell ratio. Therapy in the form of androgen depletion reversed these trends and restored helper and suppressor levels as well as the T4/T8 ratio to control values. In a small subset of sixteen patients surgery had only modest immediate (prostate) and long term (kidney) effects on these parameters.

Key words: T lymphocytes, Urologic cancer, Therapy.

Introduction

Knowledge of lymphocyte function has markedly increased in the past two decades with the description of T and B lymphocytes and the further definition of helper and suppressor T cells. Immunoregulation by means of various lymphocyte subsets is being investigated in several disease areas including allergy [1], viral infections [2], transplantation [3] and neoplasia [4]. Knowledge of lymphocytic patterns coinciding with various tumor stages and therapies could provide insight into simultaneous host-tumor immune interactions. The purpose of this report is to examine lymphocyte subsets in urologic malignancies and to further examine the relationship between these subsets and disease stages and therapy.

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Material and Methods

Patients

Ninety-one patients with a histologic diagnosis of urologic cancer as well as eight age-matched control patients without evidence of urologic cancer were evaluated (Table 1). The 91 patients consisted of 37 prostate cancer, 41 bladder cancer, and 13 kidney cancer patients.

a) Prostate Cancer. The 37 prostate cancer patients consisted of seven patients with stage A, six with stage B, nine with stage C and fifteen with stage D disease. These 37 patients were all untreated. Comparisons were made between control patients and all prostate cancer patients (Table 2), and between low stage (A and B) and high stage (C and D) disease (Table 3).

b) Bladder Cancer. The 41 bladder cancer patients consisted of 21 patients with stage A, 10 patients with stage B, 6 patients with stage C, and 4 patients with stage D disease. Comparisons were made between control patients and all bladder cancer patients (Table 4) and between low stage (A) and high stage (B, C, and D) patients (Table 5).

c) Kidney Cancer. The thirteen kidney cancer patients consisted of 6 patients with stage I, 2 patients with stage II, 2 patients with stage III, and 3 patients with stage IV cancer. Comparison were made between control patients and all kidney cancer patients (Table 6), and between low stage (I and II) and high stage (III and IV) (Table 7).

d) Therapy. An additional 26 patients were evaluated for the effect of their therapeutic intervention on lymphocyte subset distribution (Table 8). The effect of prostate cancer therapy on leukocyte subset distributions was evaluated in eight patients undergoing radical prostatectomy (Table 9) and 10 patients being treated with hormones (Table 10). The radical prostatectomy patients had post therapy samples collected a mean of seven days after surgery. The 10 patients being treated with hormones, either orchiectomy or 3 mg of diethylstilbesterol daily, and post therapy samples collected approximately three months after initiation of therapy. All patients were in clinical remission at the time of evaluation.

In addition, eight patients undergoing radical surgery and with no evidence of disease status approximately six months post surgery were evaluated (Table 11). The blood samples were collected approximately six months after the nephrectomies.

Table 1. Total urologic patients studied

Urologic cancer patients		91
1. Prostate	37	
Stage A – 7		
Stage B – 6		
Stage C – 9		
Stage D – 15		
2. Bladder	41	
Stage A – 21		
Stage B – 10		
Stage C – 6		
Stage D – 4		
3. Kidney	13	
Stage I – 6		
Stage II – 2		
Stage III – 2		
Stage IV – 3		
Control Patients		8
Total		99

Table 2. The effect of prostate cancer on lymphocyte subset distributions

	Control (n = 8)[b]	Prostate cancer (n = 37)	p[c]
OKT3[a]	69.3 ± 4.0	66.2 ± 4.9	N.S.
OKT4	38.4 ± 2.7	34.9 ± 5.1	N.S.
OKT8	20.9 ± 1.4	23.4 ± 2.3	0.01
OKM1	14.4 ± 3.0	21.0 ± 6.2	0.02
T4/T8	1.84 ± 0.14	1.51 ± 0.27	0.01

[a] Monoclonal antibodies: OKT3 (total T-cells), OKT4 (helper T-lymphocyte), OKT8 (suppressor T-lymphocyte), OKMI (monocyte, granulocytes and null cells) T4/T8 (helper/suppressor cell ratio)
[b] Number of patients
[c] P value

Monoclonal Antibodies

Monoclonal antibodies (Ortho) with the following specificities: OKT3, directed toward identification of human peripheral T-lymphocyte subclasses; OKT4 directed toward identification of human helper T-lymphocytes; OKT8, directed toward human suppressor/cytotoxic T-lymphocyte subclasses; OKM1, directed towards human monocytes, granulocytes and null cells were employed. The production and characterization of these monoclonal antibodies have been described previously [5, 6]. B lymphocyte determinations were performed using the B1 monoclonal antibody (Coulter Clone).

Table 4. The effect of bladder cancer on lymphocyte subset distributions

	Control (n = 8)	Bladder cancer (n = 41)	P
OKT3	69.3 ± 4.0	67.9 ± 2.7	N.S.
OKT4	38.4 ± 2.7	37.9 ± 3.8	N.S.
OKT8	20.9 ± 1.4	24.1 ± 3.5	0.05
OKM1	14.4 ± 3.0	23.2 ± 2.7	0.001
T4/T8	1.84 ± 0.14	1.63 ± 0.22	0.02

Isolation of Mononuclear Cells

Heparinized peripheral blood was diluted with an equal volume of RPMI 1640 and the mononuclear cells removed by ficoll-hypaque gradient centrifugation [7]. The cells were harvested with cold (4 °) RPMI 1640 or PBS, and residual red blood cells lysed by suspension in a small volume of 0.85% $(NH_4)_2Cl$. Cells were washed twice, counted by hemocytometer and diluted to 10^7 cells/ml in RPMI 1640. Viability was monitored throughout using trypan blue exclusion and was consistently found to exceed 95%. Aliquots of 0.1 ml were immediately removed for both non-specific esterase activity and monocytic, granulocytic and null cell determinations using OKM1 antibody. The remaining cells were depleted of adherent cells by incubation at 37 °C for 45 min. Non-adherent cells were collected, counted and again suspended to 10^7/ml prior to measurement of OKT3, OKT4, OKT8 and B cell reactivity.

Analysis of Peripheral Blood Mononuclear Cells

Briefly, 100 µl of the cell suspension (10^6 cells) was treated with 10 µl of the appropriate monoclonal antibody, in duplicate tubes. Controls were set up containing only PBS. Incubation was performed on ice for 15 min prior to washing with PBS at 4 °C. One hundred µl of goat-anti-mouse IgG conjugated to horseradish peroxidase (Tago, Inc.) was added and incubated on ice for an additional 15 min. Cells were washed as before and reacted at saturation with diaminobenzidine substrate for 30 min. Smears were made onto slides, air dried, washed and counterstained using 1% methyl green. Cells, which were scored positive, contained either clearly particulate brown deposits or distinct dark brown circles along the periphery of the cells. Internal staining was judged negative and corresponded to endogenous peroxidase activity. Monocytes were counted by morphology (employing Wright stain) and non-specific esterase activity [8]. Approximately 250 cells per slide from randomly select-

Table 3. The effect of prostate cancer stage on lymphocyte subset distributions

	Control	Stages A, B (n = 13)	Control vs A, B	Stages C, D (n = 24)	Control vs C, D
OKT3	69.3 ± 4.0	69.1 ± 4.8	N.S.	64.6 ± 4.3	0.02
OKT4	38.4 ± 2.7	39.9 ± 3.1	N.S.	32.4 ± 4.2	0.001
OKT8	20.9 ± 1.4	22.9 ± 2.4	N.S.	23.7 ± 2.2	0.01
OKM1	14.4 ± 3.0	16.8 ± 4.9	N.S.	23.3 ± 5.6	0.001
T4/T8	1.84 ± 0.14	1.74 ± 0.22	N.S.	1.38 ± 0.19	0.001

Table 5. The effect of stage of bladder cancer on lymphocyte subset distributions

	Control (n = 8)	Stage A (n = 21)	Control vs A	Stage B, C, D (n = 20)	Control vs B, C, D
OKT3	69.3 ± 4.0	68.4 ± 2.3	N.S.	67.1 ± 3.2	N.S.
OKT4	38.4 ± 2.7	38.8 ± 4.5	N.S.	36.5 ± 3.2	N.S.
OKT8	20.9 ± 1.4	24.4 ± 3.7	0.02	23.7 ± 3.2	0.05
OKM1	14.4 ± 3.0	23.7 ± 4.1	0.001	22.4 ± 1.8	0.001
T4/T8	1.84 ± 0.14	1.67 ± 0.24	N.S.	1.58 ± 0.17	0.001

Table 6. The effect of kidney cancer on lymphocyte subset distributions

	Control (n = 8)	Kidney cancer (n = 13)	P
OKT3	69.3 ± 4.0	66.0 ± 3.9	N.S.
OKT4	38.4 ± 2.7	37.9 ± 2.9	N.S.
OKT8	20.9 ± 1.4	24.6 ± 2.1	0.001
OKM1	14.4 ± 3.0	21.2 ± 3.3	0.001
T4/T8	1.84 ± 0.14	1.7 ± 0.17	N.S.

ed fields were counted. Results were expressed as the percentage of each T-cell subset per total number of mononuclear cells counted. Negative controls utilized to assess non-specific background staining consisted of omission of primary and secondary antibody (i.e., application of diaminobenzidine only). Internal staining was judged negative and corresponded to endogenous peroxidase. Each preparation was counted using an AO microscope and values obtained were compared using the student t test.

Statistics

The student t test was employed to compare the various groups.

Table 7. The effect of stage of kidney cancer on lymphocyte subset distributions

	Control (n = 8)	Stage I, II (n = 8)	Control vs I, II	Stage III, IV (n = 5)	Control vs III, IV
OKT3	69.3 ± 4.0	66.7 ± 4.1	N.S.	64.6 ± 3.5	N.S.
OKT4	38.4 ± 2.7	39.5 ± 3.2	N.S.	36.8 ± 2.7	N.S.
OKT8	20.9 ± 1.4	23.6 ± 1.9	N.S.	25.4 ± 2.2	0.001
OKM1	14.4 ± 3.0	20.6 ± 3.9	0.01	21.7 ± 2.5	0.001
T4/T8	1.84 ± 0.14	1.71 ± 0.16	N.S.	1.69 ± 0.19	N.S.

Table 8.

Therapy		Patients
1. Prostate cancer		18
Radical prostatectomy	8	
Hormone therapy	10	
2. Kidney cancer		8
Radical nephrectomy	8	
Total		26

Table 10. The effect of hormonal therapy on lymphocyte subset distributions

Group	Pre-therapy (n = 10)	Post-therapy (n = 10)	Pre-therapy vs post-therapy
OKT3	65.3 ± 2.6	67.9 ± 3.9	N.S.
OKT4	32.7 ± 2.7	35.0 ± 2.2	0.05
OKT8	25.1 ± 1.5	23.2 ± 1.9	0.02
OKM1	26.1 ± 4.8	22.8 ± 2.9	0.05
T4/T8	1.30 ± 0.10	1.52 ± 0.18	0.001

Table 9. The effect of radical prostatectomy on lymphocyte subset distributions

	Pre-radical prostatectomy (n = 8)	Post surgery (n = 9)	P
OKT3	62.5 ± 2.5	61.0 ± 1.3	N.S.
OKT4	39.4 ± 4.3	38.5 ± 4.6	N.S.
OKT8	22.4 ± 1.9	21.3 ± 1.7	N.S.
OKM1	21.8 ± 4.3	22.1 ± 2.2	N.S.
T4/T8	1.78 ± 0.28	1.81 ± 0.21	N.S.

Table 11. The effect of radical nephrectomy on lymphocyte subset distributions

	Pre-radical nephrectomy (n = 8)	Post-surgery (n = 8)	P
OKT3	68.1 ± 4.1	67.9 ± 3.4	N.S.
OKT4	34.9 ± 3.2	37.4 ± 2.8	N.S.
OKT8	24.2 ± 3.4	22.8 ± 3.2	N.S.
OKM1	22.8 ± 3.3	23.1 ± 3.2	N.S.
T4/T8	1.59 ± 0.27	1.68 ± 0.27	N.S.

Results

Prostate Cancer

The Effect of Prostate Cancer on Lymphocyte Subset Distributions (Table 2). Suppressor cell activity was significantly (0.01) increased (20.9 vs 23.4) in prostate cancer patients and this contributed to a significant (0.01) decrease in the T4/T8 ratio (1.84 vs 1.51).

The Effect of Stage (Table 3). Helper cell activity was significantly (0.001) decreased (38.4 vs 32.4) in the higher stage (C and D) patients and this contributed to a significant (0.001) decrease in the T4/T8 ratio (1.84 vs 1.38).

Bladder Cancer

The Effect of Bladder Cancer (Table 4). Suppressor cell activity was significantly (0.05) increased (20.9 vs 24.1) in the bladder cancer patients and this contributed to a significant (0.02) decrease in the T4/T8 ratio (1.84 vs 1.63).

The Effect of Stage (Table 5). Suppressor cell activity was significantly (0.05) increased (20.9 vs 23.7) in high stage patients and this contributed to a significant (0.001) decrease in the T4/T8 ratio (1.84 vs 1.58).

Kidney Cancer

The Effect of Kidney Cancer (Table 6). Suppressor cell activity was significantly (0.001) higher in the kidney cancer patients (20.9 vs 24.6).

The Effect of Stage (Table 7). Suppressor cell activity was significantly (0.001) higher in the high stage (III and IV) patients (20.9 vs 25.4).

Therapy

The Effect of Radical Prostatectomy (Table 9). There were no statistically significant differences between the various pre and post radical prostatectomy subsets.

The Effect of Hormone Therapy (Table 10). Helper cell activity increased (32.7 vs 35.0) and suppressor cell activity decreased (25.1 vs 23.2) after hormone therapy.

The Effect of Radical Nephrectomy (Table 11). Radical surgery had no statistically significant effect on subsets.

Discussion

Lymphocyte subsets have an immunoregulatory role in many diseases including neoplasia. Presumably, a well functioning immune system is essential to maintain host defense mechanisms against tumors, both in the form of an immune surveillance, as well as the destruction of micrometastases that express tumor associated antigens. An intact immune system is not only therapeutic but can also be prognostic; that is, lymphocytic abberations can be predictive of a poor prognosis [9].

Twenty years ago an absolute lymphocyte count could provide a degree of predictability. More recently, as additional data is generated about lymphocyte subsets and their interactions, T and B cell and helper and suppressor T cell enumerations are providing an even greater depth to our knowledge of the immune response [10].

Interest in the immune aspect of urologic tumors dates back to the early 1970's. While both humoral and cellular arms of the immune system have been studied, the latter appears more important. The cellular immune response can be either nonspecific or specific and both aspects have been evaluated. Robinson [11], McLaughlin [12], and Catalona [13] noted nonspecific cellular immunity employing T rosette and blastogenesis assays. Numerous authors have commented on microcytotoxicity [14–17] and NK cell activity [18–22] in urologic cancer patients. Specific cellular immunity to tumor extracts has been reported by Avis [23] and Bhatti [24] utilizing the leukocyte adherence inhibition assay. While the aforementioned assays were in vitro determinations, Brannen [25] skin tested prostate cancer patients with extracts of their tumors and noted a positive response in three of seven patients tested. This growing body of evidence seems to substantiate an immune response on the part of urologic cancer patients to their tumors.

The data in this report continues these previous investigations. The presence of cancer and the extent of disease correlated with subset changes in all of the urologic cancers studied. Patients with prostate, bladder and kidney cancers had lower helper and higher suppressor cells, as well as lowered T4/T8 ratios, than control patients. These changes were more pronounced in patients with higher stage disease than lower stage disease. Ritchie [26] also noted a reduced helper cell population in 32 patients with renal cell carcinoma.

Successful hormone therapy in prostate cancer patients tended to revert subset changes to control values. Previous reports regarding the effect of hormone therapy on the immune response are conflicting. Whether a beneficial result is due to the effect of the hormones themselves on the immune system or whether it is simply a systemic reflection of a decreased tumor burden are questions which remain unresolved. We have previously reported diminished leukocyte adherence inhibition following hormone [27] therapy. Employing a different parameter we now note a positive effect in patients treated with essentially the same types of hormone therapy. In all likelihood, different aspect of the immune response, as assessed by different tests, react differently to hormone therapy. The effect of surgery on these subsets is more difficult to evaluate. The effect of the operation itself (that is, the one week post surgery re-

sults) as well as the result of tumor removal (the six month post surgery results in patients with no evidence of disease) tended to return subset values to control levels. These findings are also in agreement with those of Ritchie.

The presence of cancer and the extent of the cancer seem to alter the immune response as reflected in lymphocyte subset distributions. Whether the immune system is an active participant in the therapeutic process or simply a passive responder remains a major question. This report did not address that issue. However it would seem that a return of the helper and suppressor values as well as the T4/T8 ratio to control values, at least in prostate cancer, correlated with successful hormone therapy. The consistent finding of altered lymphocytic subset distributions in patients with urologic malignancies suggests that evaluation of T-cell ratios may prove clinically valuable. As more is learned about lymphocytic activity in urologic oncology as well as other tumor systems, hopefully prognostic and therapeutic uses for these subset values will be discovered.

In summary, the presence of cancer and the increasing stage of disease are associated with a relative decrease in helper and an increase in suppressor activity as well as a decreased T4/T8 ratio. Therapy in the form of androgen depletion reversed these trends and restored helper and suppressor levels as well as the T4/T8 ratio to control values. In a small subset of sixteen patients surgery had only modest immediate (prostate) and long term (kidney) effects on these parameters.

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