Urol Res (1985) 13:67–71

Urological Research

The Effect of Hormone Therapy on Peripheral Blood Leukocytic Subset Distribution in Stage D Prostatic Cancer Patients

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Accepted: November 16, 1984

Summary. The distribution of mononuclear cell types found in the peripheral blood of patients bearing carcinoma of the prostate were compared by stage and to a control group using monoclonal antibody techniques. Patients with lower stage disease (A, B) had no significant alteration in subset distribution when compared to a control group, while those with higher stage disease (D) had significant deviations. Stage D patients had a decreased representation of helper-inducer T cells and an increased representation of suppressor-cytotoxic T cells, with an overall reduction in the total T cell content. In addition elevated levels of monocytic, granulocytic and null cells were recognized by the polyspecific OKM1 antibody. These differences were in part reversible following hormonal therapy. Such alterations in the ratios between the various T cell populations could be useful in patient staging and treatment selection.

Key words: Immune response, Prostate, Leukocytic subsets.

Introduction

The identification and quantification of the many lymphocyte subpopulations has expanded our knowlege of immune status and function. Such indicators may provide markers for monitoring immune competence in the choice of patients for surgery, radiation or chemotherapeutic treatment. The availability of monoclonal antibodies specific for the various lymphocytic subsets has facilitated the enumeration of those circulating cells potentially capable of immunologic responsiveness and regulation. A careful delineation of these populations must precede any understanding of immunopathology or possible immunotherapeutic application.

Preliminary investigations have revealed T cell imbalances in a variety of diseases including systemic lupus erythematosis, rheumatoid arthritis, infectious mononucleosis, myasthenia gravis, graft vs host disease, mycosis fungoides and leprosy [10]. The importance of the helper T cell/suppressor T cell ratio in monitoring the immune status of renal transplant patients has previously been recognized. Those transplant recipients with (helper T cell/suppressor T cell) ratios at preoperative levels and having an excess in helper T cell representation were at greater risk for organ rejection, unless the donor graft was HLA identical or the recipient had a very low total T cell content [18]. Alterations in the ratio between helper T and suppressor T cells have also been reported to occur in malignant B cell lymphomas. No such deviations were found in benign lymph nodes [17]. Similar observations have been reported by Dvoretsky [9]. Leukocytic alterations have also been reported in peripheral blood of prostatic cancer patients as well as in infiltrates of urinary tract tumors [19, 20]. More recently lowered representation by total T cells, total lymphocytes and null cell types have been correlated with poorer prognosis in a five year study of melanoma patients [5].

This study was undertaken to identify new parameters for monitoring the immune status in prostate cancer patients. Alterations in the distribution of the various cell types may also play a role in the staging of prostatic cancers, known to be difficult by conventional criteria.

Materials and Methods

Patients

For the initial study, peripheral blood was obtained from 37 patients (stage A: 7, stage B: 6, stage C: 9, stage D: 15) with prostate cancer (CAP). Staging was according to Whitmore [22] and was as follows: Stage A: clinically inapparent, Stage B: localized within the prostatic capsule, Stage C: periprostatic, and Stage D: nodal involvement or

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distant metastases. Eight age matched controls without evidence of CAP were also studied. To determine the effect of hormonal therapy upon subset distributions, ten Stage D patients were evaluated. Nine of these were new patients, not included in the early study while a single individual submitted different pre-treatment samples for inclusion in each. In most instances the blood submitted was sufficient for a complete subset evaluation.

Monoclonal Antibodies

Monoclonal antibodies (Ortho) with the following specificities were utilized: OKT3, directed towards identification of human peripheral T-lymphocytes: OKT4, directed towards identification of human helper-inducer T-lymphocytes: OKT8, directed towards human suppressor-cytotoxic T-lymphocytes: OKM1, directed towards human monocytes, granulocytes and null cells. T cell specific monoclonal antibodies were produced by immunizing mice with E rosetting lymphocytes or with thymocytes, followed by fusion of the immune spleen cells with a myeloma cell line described previously [15]. The antibodies used were obtained from the ascitic fluid of hybridoma bearing mice [23]. B lymphocyte determinations were performed using the B1 monoclonal antibody (Coulter Clone).

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 °C) 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 the OKM1 antibody. The remaining cells were depleted of adherent cells by incubation at 37 °C for 45 min in plastic tissue culture dishes (Falcon). 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

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 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 identified by morphology (employing Wright stain) and non-specific esterase activity [14]. Each preparation was counted using an AO microscope and values obtained. Means and standard deviations were determined for each group, and the student t test utilized to determine degree of significance.

Hormonal Therapy

To determine the effect of hormonal manipulations on leukocytic subset distributions, ten stage D patients were selected for evaluation. Blood samples were obtained, from each individual, *pre-* and at least three weeks *post-*hormonal therapy and values compared using a two tailed self paired analysis. Pre treatment values were also compared to the control group using the student t test as described above. Hormonal therapies consisted of a variety of treatment protocols which included administration of various LH-RH agonists, DES or orchiectomy.

Results

Using the monospecific antisera the following distribution of the various cell types was observed in control subjects. Sixty-nine percent of the non-adherent peripheral blood mononuclear cells were T cells by OKT3, 38% were helper-inducer T cells by OKT4, 21% suppressor-cytotoxic T cells by OKT8 and 8.5% B lymphocytes (Table 1). Monocytic representation, determined morphologically and by non-specific esterase activity, showed a 10% contribution within the peripheral blood. In addition reactivity with the poly-specific OKM1 antibody revealed that 14% of the cells in the circulation were monocytes, granulocytes or null cells.

Table 1. Distribution of monoculear cells in control, low stage (A&B), and high stage (C&D) patients

Group	Control	Stages (A, B)	% Change from control	Significance	Stages (C, D)	% Change from control	Significance
OKT3[a]	69.3 ± 4.0	69.1 ± 4.8	0	n.s.	64.6 ± 4.3	7	< 0.02
OKT4[a]	38.4 ± 2.7	39.9 ± 3.1	3	n.s.	32.4 ± 4.2	16	< 0.001
OKT8[a]	20.9 ± 1.4	22.9 ± 2.4	9	n.s.	23.7 ± 2.2	13	< 0.01
OKM1[b]	14.4 ± 3.0	16.8 ± 4.9	16	n.s.	23.3 ± 5.6	62	< 0.001
B Cells[a]	8.5 ± 2.0	7.3 ± 2.8	14	n.s.	9.7 ± 3.3	14	n.s.
Monocytes[b]	10.1 ± 3.0	10.4 ± 3.2	2	n.s.	14.9 ± 3.6	48	< 0.01
T3/T4	1.81 ± 0.08	1.76 ± 0.08	3	n.s.	2.01 ± 0.18	11	< 0.01
T3/T8	3.33 ± 0.19	3.05 ± 0.35	8	n.s.	2.78 ± 0.34	17	< 0.001
T4/T8	1.84 ± 0.14	1.74 ± 0.22	5	n.s.	1.38 ± 0.19	25	< 0.001

[a] Expressed as % of total non-adherent cells
[b] Expressed as % of total mononuclear peripheral blood cells

Table 2. Distribution of mononuclear cells in control, stage C, and stage D patients

Group	Control	Stage C	% Change from control	Significance	Stage D	% Change from control	Significance
OKT3[a]	69.3 ± 4.0	65.7 ± 4.7	5	n.s.	64.0 ± 4.2	8	< 0.02
OKT4[a]	38.4 ± 2.7	34.7 ± 4.5	10	n.s.	31.1 ± 3.5	19	< 0.001
OKT8[a]	20.9 ± 1.4	23.0 ± 2.0	10	< 0.05	24.1 ± 2.3	15	< 0.01
OKM1[b]	14.4 ± 3.0	20.0 ± 5.3	39	< 0.05	25.3 ± 4.9	76	< 0.001
B Cells[a]	8.5 ± 2.0	10.4 ± 4.5	23	n.s.	9.2 ± 2.3	8	n.s.
Monocytes[b]	10.1 ± 3.0	13.7 ± 4.3	35	n.s.	15.7 ± 2.9	55	< 0.001
T3/T4	1.81 ± 0.08	1.91 ± 0.16	6	n.s.	2.08 ± 0.16	15	< 0.001
T3/T8	3.33 ± 0.19	2.95 ± 0.41	11	< 0.05	2.68 ± 0.24	20	< 0.001
T4/T8	1.84 ± 0.14	1.51 ± 0.21	18	< 0.01	1.29 ± 0.13	70	< 0.001

[a] Expressed as % of total non-adherent cells
[b] Expressed as % of total mononuclear peripheral blood cells

Table 3. The effect of hormonal therapy on leukocytic subset distribution

Group	Control	Pre-therapy	Post-therapy	Pre-therapy VS Post-therapy	Pre-therapy VS control
OKT3[a]	69.3 ± 4.0	65.3 ± 2.6	67.9 ± 3.9	n.s.	< 0.05
OKT4[a]	38.4 ± 2.7	32.7 ± 2.7	35.0 ± 2.2	< 0.05	< 0.001
OKT8[a]	20.9 ± 1.4	25.1 ± 1.5	23.2 ± 1.9	< 0.02	< 0.001
OKM1[b]	14.4 ± 3.0	26.1 ± 4.8	22.8 ± 2.9	< 0.05	< 0.001
B Cells[a]	8.5 ± 2.0	8.1 ± 2.6	7.8 ± 2.4	n.s.	n.s.
Monocytes[b]	10.1 ± 3.0	18.6 ± 5.6	15.3 ± 5.2	n.s.	< 0.01
T3/T4	1.81 ± 0.08	2.01 ± 0.19	1.94 ± 0.13	n.s.	< 0.05
T3/T8	3.33 ± 0.19	2.61 ± 0.17	2.95 ± 0.32	< 0.01	< 0.001
T4/T8	1.84 ± 0.14	1.30 ± 0.10	1.52 ± 0.18	< 0.001	< 0.001

[a] Expressed as % of total non-adherent cells
[b] Expressed as % of total mononuclear peripheral blood cells

A significant pattern for leukocytic subset alterations was observed when patients were grouped by stage(s) prior to comparison with the control group. If patients are grouped into those bearing lower (A, B) or higher (C, D) stage disease, those with lower stage had no significant deviations from established control values whereas those with higher stage had large differences (Table 1).

Data compiled from the 24 patients having stage C or D disease showed a 7% decrease in total T cell content and a 16% decrease in the helper-inducer T cell subset. A 13% increase in suppressor-cytotoxic T cell content resulted in an 11% increase in the total T cell/helper-inducer T cell ratio, a 17% decrease in the total T cell/suppressor-cytotoxic T cell ratio and a 25% decrease in the helper-inducer/suppressor-cytotoxic T cell ratio. Monocytic contributions determined by non-specific esterase activity were elevated by 48%. Monocytic, granulocytic and null cell contributions measured by OKM1 were elevated by 62%.

If the higher stage patients were broken down separately into stages C and D, most of the deviations from normal values found for stages C and D combined were contributed by those in stage D (Table 2). Stage C patients had a significant 10% increase in suppressor-cytotoxic T cell content. This increase resulted in an 11% decrease in total T cell/suppressor-cytotoxic T cell ratio and an 18% decrease in helper-inducer/suppressor-cytotoxic T cell ratio. Stage C patients also had a 39% increase in those cells reacting with OKM1, representing monocytic, granulocytic and null cell types. Greatest deviations from the control values can be seen in those patients with stage D disease. They had an 8% decrease in total T cell number and a 19% decrease in helper-inducer T cell content. Stage D patients had a 15% increase in total T cell/helper-inducer T cell ratio and decrease of 20% and 70% in the total T cell/suppressor-cytotoxic and helper-inducer/suppressor-cytotoxic T cell ratios respectively. Monocytic contributions in stage D patients were elevated 55% by non-specific esterase analysis and OKM1 positive cells were up by 76%.

The final table (Table 3) demonstrates the effect of hormonal therapy on peripheral blood subset distribution in ten Stage D prostate cancer patients. These data indicate that: 1) When these ten stage D patients are evaluated and compared to control values established in the initial study, significant and comparable deviations are once again found,

therefore confirming the previous observation of altered subset distributions in those having advanced disease. 2) Hormonal therapy has a significant effect upon the helper-inducer and suppressor-cytotoxic T cell populations as well as upon monocytic, granulocytic and null cell content. 3) With the exception of the B cell population, all post-therapy subset values are intermediate between control and pre-treatment, Stage D values. 4) The population which appears most sensitive to hormonal therapy is the suppressor-cytotoxic, OKT8 reactive cell type. This is illustrated not only by the level of significance between pre and post therapy samples but also by its significant shift in ratio with the total T cell content and with the helper-inducer T-cell subset. Due to the variety of treatments utilized and the limited number of patients examined, the individual effects of each were not identified.

Discussion

Adenocarcinoma of the prostate is the second most common carcinoma occurring in American men and accounts for the third greatest total of male cancer deaths. Due to inherent problems associated with staging, it has been estimated that 50% of those patients diagnosed clinically as having stage C disease may ultimately be classified as stage D, because of nodal involvement. The identification of immunologic markers, such as the distribution within the blood of the various immunologically responsive cell types, could play a role in staging or treatment.

The data from this investigation indicate that patients having advanced prostate cancer particularly stage D, have altered mononuclear cell distributions. Total T-cell content is decreased and the ratio between helper-inducer and suppressor-cytotoxic subsets is altered. These patients also have increased numbers of monocytic, granulocytic and null cell types, while patients with less extensive disease (A, B) have no significant deviations from control values. Those alterations may indicate a lack of immunologic responsiveness directed towards tumors which remain encapsulated. Indeed several investigators have postulated that the prostate may represent a privileged site, its anatomical location and structure preventing routine immunologic surveillance [11]. In this regard Ablin et al. have shown that seminal fluid possesses immunosuppressive qualities and could play a role in the development and relatively high incidence of prostatic cancer [3].

Previous investigators have shown that patients bearing solid tumors have alterations in their distribution of T-cells but such data remain controversial. It has been estimated that at any time less than 1% of the total lymphocytes are in transport and that any differences detected may only represent lymphatic redistribution [21]. In this study, the large number of samples examined and their increasing deviation from normal with advancing stage would refute this. Other criticisms may be directed at the fact that the patient population being studied is more elderly than those included in many other immunologic surveys. These are questions which are inherent to any population prone to diseases associated with old age in which there is an increased incidence of malignancy and increased rates in the development of autoimmunity.

Hormonal therapy has been shown to suppress immunologic responsiveness of patients with prostate cancer [1, 2, 4, 12, 13, 16]. Recent studies from this laboratory have further demonstrated that patients who received hormonal therapy had significantly increased levels of circulating immune complexes in their sera; whereas, patients treated by transurethral resection showed no such increases [6].

Why hormonal therapy produces significant reduction in the suppressor-cytotoxic T-cell representation is not known. One plausible theory would assume that, after the tumor load has been reduced by therapy, suppressive tumor derived factors, directed at mononuclear peripheral blood cells, sufficient initially to cause deviations, are no longer as effective or sufficient to continue to alter subset distributions. A larger study might show increasingly significant shifts in additional populations. In fact, increasing tumor burdens in more advanced cancers may explain why significant changes are only found in higher stage patients. Correlation between altered representation by OKT8 positive cells (suppressor-cytotoxic) and changes in levels of cytotoxic activity was not determined.

Alternatively, the presence of estrogen receptors on suppressor T-cells (OKT8) may provide insight into the effect of hormonal therapy on the reduction of this subpopulation [8].

At present, we must develop faster and more efficient procedures for the early detection, staging, and treatment of prostatic carcinoma. The identification of immunologic markers could contribute to such a role in management of this potentially treatable disease.

Acknowledgement. This investigation was supported in part by the Blum-Kovler Foundation.

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