

ENDOGENOUS AND EXOGENOUSLY-INDUCED IMMUNOMODULATION OF TUMOUR-HOST RESPONSIVENESS

RICHARD J. ABLIN * / +, JANOS POLGAR ** & MAURICE J. GONDER *

* Department of Urology, Health Sciences Center, SUNY at Stony Brook, Stony Brook, NY 11794-8093, USA ** Department of Clinical Chemistry, University School of Medicine, 4012 Debrecen, Hungary

In spite of the availability of multiple effector mechanisms of the immune system to combat tumour growth and metastases, their impairment frequently accompanies the appearance of cancer. Factors contributing to this impairment may be related to properties of the host and/or the tumour itself and may be with respect to their origin -endogenous or exogenous. Based on the unique biological behavior of prostate cancer (PCa), and its apparent escape from immune surveillance in the presence of tumour immunogenicity, continuing investigation of endogenous and exogenous factors thought to be relevant to its pathogenesis have been made. For this purpose further studies of the suggested role of human seminal plasma (SePI) and the synthetic oestrogen, diethylstilboestrol (DES), as representative endogenous and exogenous immunomodulatory factors (IMF) of tumour-host responsiveness, together with evaluation of human prostatic tissue extracts and leuprolide (the luteinizing-hormone-releasing-hormone proposed as an alternate to DES therapy) have been made by evaluating their effect on the lytic activity of natural killer (NK) cells. SePI and prostate extracts significantly suppressed NK cell lysis. Physicochemical studies suggest SePI and prostate IMF to be associated with high and low molecular weight macromolecules; and implicate the participation of transglutaminase and prostaglandins. Comparative study of therapeutic levels of DES vs. leuprolide on NK cell lysis demonstrated significant suppression by DES vs. a negligible effect of leuprolide. Metastases are highly prevalent in PCa, and contribute significantly to its morbidity and mortality. Further knowledge of the range of effects of endogenous and exogenous IMF on effector mechanisms of tumour-host responsiveness, to include suppression of NK cells, and elucidation of their nature, may contribute toward our understanding of the unique biological behavior of tumours of the prostate, in addition to improvement in their clinical management.

Multiple effector mechanisms of the immune system participate in host resistance to the growth of tumours and their metastases. However, impairment of immune responsiveness frequently accompanies the appearance of cancer. The mechanisms(s) responsible for immune suppression, and by which cancer cells avoid destruction are not fully understood. In general, factors involved in the escape of tumours from immunological destruction, and their subsequent dissemination (metastasization), may be related to properties of the host and/or of the tumour itself.

Certainly not specifically defined by the above, but characterized by a unique biological

behavior suggestive of host and tumour induced immunomodulatory factors (IMF) in its pathogenesis, is that of prostate cancer (PCa). This unique, perhaps best described as, 'antithetical behavior', is exemplified by the findings that: greater than 70% of PCa patients have metastases on diagnosis of their primary prostatic tumour, -yet, for every patient who develops clinical PCa, there are more than a hundred who have latent Ca, and do not develop clinical Ca during their lifetime (Tulinius, 1982). Further representation of this antithesis is, perhaps seen in the wide difference in the geographical incidence of mortality from clinically expressed PCa, e.g., USA vs. Japan, to the almost identical incidence of latent PCa found on autopsy in the USA and Japan. This diversity in natural history and wide variation in age of onset of clinical disease "places a total population of 95 million in the category 'at risk' for PCa" (Griffiths, 1982). It would appear some mechanisms(s) regulate the clinical expression of PCa.

By no means to be discounted in consideration of host responsiveness to the prostate, is -benign prostatic hypertrophy (BPH). BPH af-

+ To whom correspondence must be sent.

These studies were supported in part by Biomedical Research Support Grant RR05736 from the National Institute of Health and by funds to Doctor Janos Polgar from the International Cancer Research Data Bank Programme of the National Cancer Institute, National Institutes of Health (US), under contract No. NO1-CO-65341 (International Cancer Research Technology Transfer -ICRETT) and partly by the International Union Against Cancer.

fects approximately 50% of all males over age 50 (Birkhoff, 1983). Together with PCa, BPH represents an increasingly significant medical problem for the aging male.

Prostate tissue-specific and putative tumour-associated antigens and their epitopes have been identified (Ablin, 1985 a). In spite of this, and evidence of immunological responsiveness to tumor in patients with BPH (Ablin, 1983a; Ablin & Gonder, 1985a), and PCa (Ablin & Bhatti, 1981; Ablin & Gonder, 1985a), these responses appear to be less than in patients with other solid tumours.

Escape from immune surveillance in the presence of tumour immunogenicity may lie with factors within the tumour, i.e., its microenvironment (milieu) (Ablin, 1977). Homeostasis (equilibrium) between the host and tumours of the prostate, characterized by diversity in natural history, wide variation in the age of onset of clinical disease and tumour heterogeneity, may be reflective of endogenous IMF. Equally significant to this equilibrium are exogenously-induced immunological alterations acquired via therapy (Ablin & Gonder, 1985a).

Initial investigation of the contributory role of components of the prostatic secretory milieu as possible endogenous IMF demonstrated immunosuppression (IS) of humoral and cell-mediated tumour-associated immunity (TAI) in PCa patients by prostatic and seminal fluids (seminal plasma (SePI) (Ablin et al., 1980). Following a lead from earlier studies suggestive of the presence of transglutaminase (TGase)-like activity in the rabbit prostate and secretions (Bronson et al., 1970), preliminary identification of TGase as a possible contributory factor to human SePI IS has been made (Ablin & Gonder, 1985b).

In aligned studies, evaluation of exogenously-induced alterations of tumour-host responsiveness have involved the effect of the synthetic oestrogens, i.e., diethylstilboestrol (DES) and diethylstilboestrol-diphosphate (DES-P), used for the treatment of metastatic PCa, on immunologic responsiveness. These studies demonstrated the IS effects of oestrogen on mitogen- (Ablin et al., 1976; Haukaas et al., 1982) and antigen- (Ablin et al., 1979; Ablin, 1981a) induced cell-mediated immunologic responsiveness, and alterations in the subset distribution of mononuclear cells (Rubenstein et al., 1985), in patients with PCa.

The above provides presumptive evidence of IMF possibly relevant to tumours of the prostate. As indicated, metastases are highly prevalent in PCa, and contribute significantly to its morbidity and mortality. The multiplicity of host effector mechanisms suggest no single mechanisms may be responsible for antitumour immunity. Nonetheless, natural killer (NK) cells have been demonstrated to be of major importance in immune surveillance against cancer, particularly in the control of metastases (Herberman, 1982). As such, in continuing investigation of the role of SePI and DES, as representative endogenous and exogenous IMF, their effect, in addition to that of extracts of human prostate, and leuprolide (Lupron) (one of the recently suggested synthetic peptide analogues of the luteinizing-hormone-releasing-hormone (LHRH) proposed as an alternate to DES therapy (The Leuprolide Study Group, 1984) on the lytic activity of NK cells has been investigated. Inherent in this evaluation have been studies directed toward the biochemical characterization and identification of these IMF.

MATERIALS AND METHODS

Endogenous Factors

Seminal Plasma (SePI) - Human SePI clarified free of spermatozoa was obtained from ejaculates of males attending a fertility clinic. When possible, ejaculates were collected in vessels containing 1 mM phenylmethylsulfonyl fluoride (PMSF) and ethylenediamine tetraacetic acid (EDTA) to prevent polymerization of TGase. Pools of SePI (comprised of ≥ 6 donors) were formed on the basis of sperm quality as defined by: sperm count (density), motility and morphology.

Pool 003, the results of which are presented, was comprised of 23 specimens with spermatozoa counts $< 40 \times 10^6$ /ml which had been stored for 2 years at -20°C . This pool had a protein concentration of 18.1 mg of protein/ml as determined with the Coomassie blue protein assay of Bradford (1976) using bovine gamma globulin as standard.

Prostate Extracts - Extracts were prepared from human prostate tissue obtained at surgery from patients with BPH and PCa and was stored at -20°C . Weighed tissues were pooled and extracted at 1:3 (weight/volume) in 50 mM Tris-HCl buffer, pH 7.5, with 0.25 M sucrose;

1 mM PMSF and EDTA at 4°C. Homogenates were stirred and re-homogenized and centrifuged at 31,000 xg for 1 hr. at 4°C. Protein concentrations of benign prostate extracts (BPE) ranged from 14.1 to 23.9 mg of protein/ml by the Coomassie blue protein assay. The protein concentration of PCa extract (PCaE), of which there was one pool available for study, had a protein concentration of 12.0 mg/protein/ml.

Exogenous Factors

Diethylstilboestrol (DES) – DES (Sigma Chemical Company, St. Louis, MO, Lot No. 49C-0092) was dissolved in dimethyl sulfoxide (DMSO, Fisher Scientific Company, Springfield, NJ) at a final concentration of 0.05-0.5%.

Leuprolide – Leuprolide acetate (Lupron, courtesy of TAP Pharmaceuticals, North Chicago, IL, Lot No. 39-102-AL) was reconstituted in RPMI-1640 medium (Grand Island Biological Company, Grand Island, NY).

Effector Cells

Heparinized blood was obtained by Informed Consent from patients and healthy adult volunteers. Peripheral blood mononuclear cells (PBMC) were obtained by density gradient centrifugation of diluted blood on Histopaque-1077 (Sigma Chemical Company, St. Louis, MO). PBMC were collected, washed and resuspended to appropriate concentrations as needed in RPMI-1640 medium supplemented with 10% heat-inactivated foetal calf serum containing 4 mM L-glutamine, 100 units/ml penicillin G and 100 µg/ml streptomycin.

In selected studies, PBMC were further depleted of adherent cells (monocytes) by incubation in a sterile plastic tissue culture flask for 60 min. at 37°C with 5% CO₂ in air.

As PBMC from the same donor were evaluated untreated and treated, each donor served as their own control.

Assay for Natural Killer Cell Activity

Cytotoxicity of PBMC for the human K-562 erythroleukemia tumour cell line, used as the target cells, was evaluated, at a 50:1 effector to target (E:T) cell ratio (determined optimal from evaluation of E:T cell ratios ranging from 100:1 to 12.5:1) in a 4 hr. microcytotoxicity assay using ⁵¹Cr (New England Nuclear, Boston, MA) modified after the method of Brunner et al. (1968). The mean value of counts per minute (cpm) of triplicate cultures was used for statistical evaluation. NK cell activity, i.e., the per-

cent specific release of ⁵¹Cr (cytotoxicity was determined as:

$$\% \text{ Specific Cytotoxicity} = \frac{\text{Experimental cpm} - \text{Spontaneous cpm}}{\text{Maximum cpm} - \text{Spontaneous cpm}} \times 100$$

Where, spontaneous release (control cultures) consisted of ⁵¹Cr-labeled target cells incubated in assay medium only. Maximum release consisted of ⁵¹Cr-labeled target cells incubated in 10% sodium dodecyl sulfate.

Evaluation of Immunomodulatory Factors (IMF)

The effect of endogenous and exogenous IMF -SeP1 and BPE and DES and leuprolide, respectively, on the lytic activity of NK cells was evaluated and compared. For this purpose, PBMC from the same donor were preincubated in cultures of RPMI-1640 medium *only* (untreated) and in RPMI-1640 medium containing the optimal inhibitory concentration (determined from a dose-response curve) of the respective endogenous or exogenous IMF, for 18 hrs. at 37°C in 5% CO₂ in air. Following incubation, cells were washed 3 times in RPMI-1640 medium and the respective cell viabilities determined by trypan-blue dye exclusion. The lytic activity of untreated and treated PBMC was then determined by the NK cell assay described above.

Transglutaminase (TGase) Activity

The TGase activity of SeP1, BPE and PCaE was evaluated by measuring the incorporation of ³H-putrescine (New England Nuclear, Boston, MA) into Hammersten casein (United States Biochemical Corporation, Cleveland, OH) by a modification of the method of Lorand et al. (1972) as detailed elsewhere (Ablin et al., 1987). Purified bovine FXIII and guinea pig liver TGase (the latter, courtesy of Dr. Soo II Chung) served as controls. The mean value of duplicate determinations of the incorporation of putrescine, expressed as disintegrations per minute, was represented as picomoles of ³H-putrescine incorporated/min/mg protein (pmol.min⁻¹.mg⁻¹).

Characterization of Endogenous Immunomodulatory Factors (IMF)

Initial evaluation toward characterization of IMF of SeP1 and BPE was made by dialysis, gel filtration and treatment with iodoacetamide, indomethacin and anti-FXIII a.

For dialysis and gel filtration studies, SeP1 and BPE were either dialyzed overnight at 4°C

against distilled water using cellulose tubing with molecular weight (M_r) exclusions of 1 and 10 Kd (Spectrum Medical Industries, Los Angeles, CA), or passed through a Sephadex G-25M (Pharmacia, Piscataway, NJ) column.

Treatment with iodoacetamide, indomethacin or anti-FXIII α (Calbiochem, LaJolla, CA, Lot No. 105814D) was accomplished by incubation of SeP1 and BPE at 37°C for 1 hr. without and with various concentrations of the respective inhibitors. Iodoacetamide treated preparations were further dialyzed or gel-filtrated (same conditions as given above).

The IM activity of untreated, and SeP1 and BPE treated preparations, was then determined by their respective incubation with PBMC and evaluation in the NK cell assay described above.

Untreated, and SeP1 and BPE treated with iodoacetamide and anti-FXIII α (BPE only), were concomitantly evaluated for their TGase activity.

Fractionation Studies

Molecular sieve chromatography of SeP1 and BPE was carried out at 4°C on Sephadex G-200 and Sephacryl S-300 (Pharmacia, Piscataway, NJ) columns. The columns were equilibrated and eluted with PBS, pH 7.2. The relative absorbance of each fraction was measured at 280 nm using a Uvicord SII UV monitor (LKB, Bromma, Sweden) with a 2.5 x 5 mm measuring cell. The M_r of the fractions were estimated by a mixture of thyroglobulin (670 Kd); gamma globulin (158 Kd); ovalbumin (44 Kd); myoglobin (17 Kd) and Vitamin B-12 (1.35 Kd) (Bio-Rad Laboratories, Richmond, CA).

Individual fractions and small pools of selected fractions were concentrated 50 x by ultrafiltration (Minicon-B15 Concentrator, M_r exclusion 15 Kd, Amicon Corporation, Danvers, MA) for evaluation of TGase activity.

Statistical Analysis

The Student's "t" test was used to determine the statistical significance of the data. In the case of concomitant independent incubation of PBMC with endogenous or exogenous IMF or their treatment thereof, e.g., with iodoacetamide, this was by the paired "t" test. In the absence of concomitant incubation, comparison was made by the "t" test on two independent means. The variability of the data is presented as the mean \pm SD.

RESULTS

The observations of this study are presented according to the evaluation of the endogenous and exogenous IMF of SeP1 and BPE and DES and leuprolide, respectively, with reference to their *in vitro* effect on the lytic activity of NK cells. In the case of SeP1, BPE and PCaE initial results obtained toward characterization and identification of their IMF are further presented.

The temporal sequence of specimen availability and variability in the number of PBMC precluded concomitant evaluation of both endogenous and exogenous IMF in a number of the experiments.

Evaluation of Endogenous Immunomodulatory Factors

Preparations of SeP1 (Pool 003) and BPE (1/2) were evaluated as endogenous IMF on the basis of their respective effects on the lytic activity of PBMC for K-562 target cells.

Treatment of PBMC with SeP1 and BPE resulted in a reduction of their lytic activity compared to that obtained with untreated cells. Reduction of NK cell lysis was proportional to the concentration of SeP1 and BPE. In the case of SeP1, variable reductions occurred between 100 and 1000 μ g/ml and with BPE between 100 and 500 μ g/ml. As the percent reduction of lysis for SeP1 treated PBMC appeared maximal at 1000 μ g/ml, this was taken as the optimum inhibitory dosage. In the case of BPE, 250 μ g/ml was selected as optimal, as occasional lysis of PBMC, noted from pre-test cell viability counts of only 65-80%, were noted at 500 μ g/ml.

The percent reduction in lysis of individual specimens of PBMC treated with 1000 μ g/ml SeP1 ranged from 36 to 99%, and with 250 μ g/ml BPE from 64 to 100% from that of concomitantly evaluated untreated PBMC.

A summary of the respective effects of SeP1 and BPE on the lytic activity of NK cells is presented in Table I. Treatment of PBMC with SeP1 and BPE resulted in highly significant mean reductions in lysis of 63 ($P < 0.01$) and 80% ($P < 0.001$), respectively.

The possibility the observed reduction in lysis was due to a cytotoxic effect of SeP1 or BPE was initially thought to be excluded by evaluating the viability (as assessed by trypan-blue dye exclusion) of PBMC incubated for 18 hrs. in culture medium with and without their

TABLE I

Summary of the effect of human seminal plasma (HSePl) and benign human prostate extract (BPE) on the lytic activity of natural killer cells

Peripheral blood mononuclear cells	Percent lysis of K-562 target cells (Mean \pm SD)*	Percent reduction in lysis from untreated	Significance (P) of percent reduction in lysis from untreated
Peripheral			
:: Untreated	48.0 \pm 14.7 (10)**	—	—
Treated with:			
1000 μ g/ml*** HSePl (Pool 003)	17.7 \pm 17.8 (5)	-63	<0.01
250 μ g/ml*** BPE (1/2)	9.6 \pm 8.2 (5)	-80	<0.001

*Determined from triplicate determinations on each donor at an effector: target cell ratio of 50:1.

**Number of donors evaluated.

***Optimum inhibitory dosage as determined from a dose-response curve ranging from 10-1000 μ g/ml for HSePl and 1-500 μ g/ml for BPE.

respective optimal inhibitory dosages. Cell viability from such evaluations ranged from 85 to 92%.

Occasional loss of cell viability, initially noted with PBMC treated with BPE at 500 μ g/ml (as mentioned earlier) prompted additional study of a possible cytotoxic effect as contributory to reduced NK cell lysis. Further included in this study, from that delineated above, was the temporal evaluation of cell viability of untreated and SePl and BPE treated PBMC up to 24 hrs. the time corresponding to that from the initial separation of PBMC to completion of the NK cell assay. These studies demonstrated that IS by SePl and BPE could be differentiated from a lymphocytotoxic effect of an aminoaldehyde ('acrolein') arising from the oxidation of SePl and prostate polyamines by amine oxidase (present in the foetal calf serum supplement used) by pretreatment of SePl and BPE with 0.2 mM hydroxylamine (an amine oxidase inhibitor).

Comparable significant differences in the respective effects of SePl and BPE on NK cell lysis were observed with adherent cell (monocyte) depleted PBMC.

Studies directed toward elucidating the mechanism of action of IMF in SePl and BPE suggest they function at the level of the effector, rather than target cell.

The results of assays representative of the identification of TGase in SePl and BPE are presented in Table II.

The TGase activity of SePl was Ca^{2+} , but not thrombin dependent and was inhibited by iodoacetamide.

In the case of BPE, TGase activity was thrombin and Ca^{2+} dependent, inhibited by iodoacetamide and depleted by treatment with anti-FXIII a.

In contrast to prostate TGase, the Ca^{2+} requirement of SePl TGase appeared to be provided, as explained in Table II, by endogenous Ca^{2+} .

The presumptive relationship between in SePl and BPE and TGase is illustrated in Table III. For this purpose, SePl and BPE were compared untreated and following treatment with 10 and 100 mM iodoacetamide for their IS and TGase activity.

As shown in Table III, treatment with 100 mM iodoacetamide virtually inhibited all TGase activity in SePl and BPE. The loss in TGase activity correlated with the respective reduction in the IS activity of SePl from 82 to 31 and 5%, and with BPE, from 66 to 6 and 5%. As noted in Table III, there was a lesser effect on the IS and TGase activity of SePl vs. BPE treated with 10 mM iodoacetamide.

Pretreatment of SePl with 0.2 mM hydroxylamine (to abrogate the formation of 'acrolein') did not significantly affect the relationship between its IS and TGase activity.

In view of the suggested relationship between IS and TGase activity, it is noteworthy, as shown in Table IV, that evaluation of PCaE

TABLE II

Representative results identifying transglutaminase activity in human seminal plasma (HSePl) and benign human prostate extract (BPE)

Additions, deletions and treatments	Transglutaminase activity in: ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	
	HSePl (Pool 003)	BPE (Pool 2)
Complete System*	3.6	6.3
Minus:		
Thrombin	3.3	0.8
Calcium**	3.2	1.0
Thrombin and calcium	3.3	0.4
Treated with:		
Iodoacetamide		
10mM	1.3	0.2
100 mM	0.8	0.4
Anti-FXIII a	—***	0.3

*In accord with assay conditions given under "Materials and Methods."

**Value obtained suggests HSePl TGase not Ca^{2+} dependent. However, the presence of endogenous Ca^{2+} in HSePl was sufficient for enzyme activity. This may be demonstrated by the addition of EDTA to HSePl in the absence of CaCl_2 , as a source of Ca^{2+} , wherein there was a decrease in TGase activity.

***Not performed.

TABLE III

Relationship between immunomodulatory factor(s) in human seminal plasma (HSePl) and benign human prostate extract (BPE) and transglutaminase activity

Immunomodulator	Treatment of immunomodulator	Transglutaminase activity ($\mu\text{mol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)	Percent lysis of K-562 target cells (Mean \pm SD)*	Percent change of lysis from control**
None (Control)**	—	—	58.6 \pm 16.4(3)***	—
HSePl (Pool 003)	—	3.6	10.8 \pm 15.0(3)	—82
1000 $\mu\text{g}/\text{ml}$	10mM	1.3	40.7 \pm 2.8(2)	—31
	100mM	0.8	55.4 \pm 11.7(2)	—5
	Iodoacetamide + Dialysis			
None (Control)	—	—	40.2 \pm 7.9(2)	—
BPE (Pool 2)	—	6.3	13.8 \pm 3.5(2)	—66
250 $\mu\text{g}/\text{ml}$	10mM	0.2	38.0 \pm 8.2(2)	—6
	100mM	0.4	38.1 \pm 21.4(2)	—5
	Iodoacetamide + Dialysis			

*Determined from triplicate determinations on each donor at an effector: target cell ratio of 50:1.

**Peripheral blood mononuclear cells not treated with HSePl or BPE.

***Number of donors evaluated.

(of which its IM properties are under investigation) disclosed significantly higher levels of TGase than BPE. As with BPE (Table II), TGase present in PCaE was thrombin and Ca^{2+} dependent and inhibitable by anti-FXIII a.

Initial characterization of the macromolecular components of SePl and BPE were made by fractionation on Sephadex G-200 and Sephacryl S-300. A representative chromatogram of the Sephadex G-200 fractionation of BPE, and

the M_r of three principal peaks, designated 1-3, are shown in Figure 1.

Sephadex G-200 fractionation of SePl (not shown) disclosed a similar chromatographic pattern with three principal peaks of M_r similar to that of BPE.

The major difference between BPE and SePl was in the qualitative differences in the relative distribution of the three peaks. Particularly stri-

king, was the suggestion of a greater amount of low M_r material (Peak 3) in SeP1 vs. BPE.

Evaluation of fractions of SeP1 and BPE for TGase, permitted, as further shown in Figure 1 for BPE, localization of the principal areas of activity. These corresponded to M_r of 90-100 Kd and 50-60 Kd.

The principal areas of TGase activity in SeP1 (not shown) corresponded to M_r of 90-100 Kd and 20-30 Kd.

TABLE IV

Activity of transglutaminase in extracts of benign and malignant human prostate

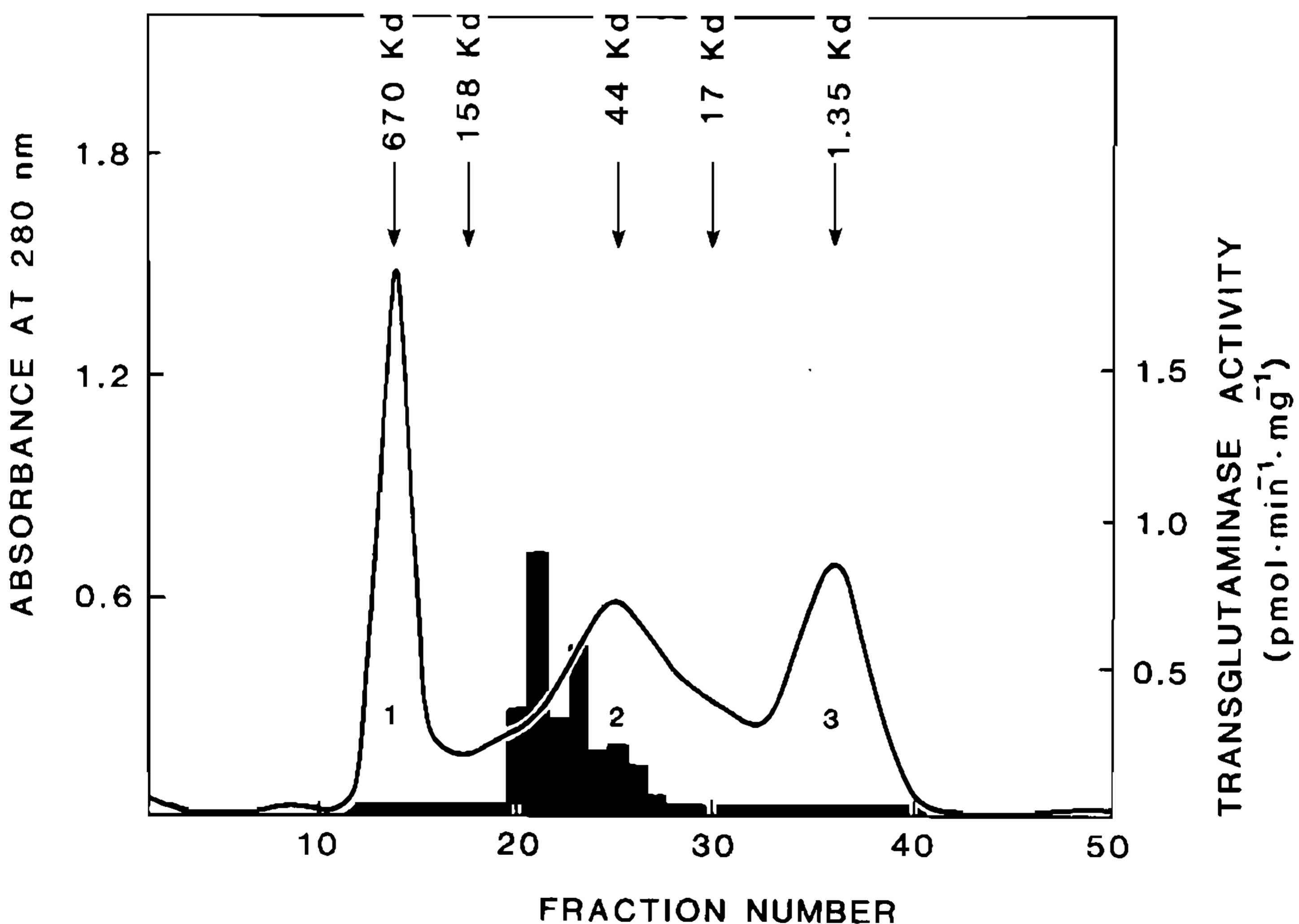
Source of human Prostate tissue	Transglutaminase activity with: ($\text{pmol} \cdot \text{min}^{-1} \cdot \text{mg}^{-1}$)		
	Thrombin	Without Thrombin	Thrombin and anti-FXIII _a
Benign	6.3	0.8	0.3
Malignant	42.2	5.8	4.7

Subsequent fractionation of SeP1 on Sephacryl S-300 permitted delineation of four principal peaks. Peak 4, the lowest of the M_r fractions, appeared to be a further separation of the low M_r materials seen in Peak 3 on Sephadex G-200.

Evaluation toward characterization of the low M_r fractions of SeP1 and BPE (designated Peak 3 in Figure 1) was made by exclusion dialysis, gel-filtration and treatment with indomethacin.

Following an experimental format similar to that shown in Table III, dialysis and gel-filtration suggested a greater loss of IMF with BPE than SeP1 (data not shown).

Treatment with 1 and 10 μM indomethacin had an opposite effect on SeP1 and BPE. SeP1 treated with indomethacin exhibited an appreciable loss of IMF. In contrast, indomethacin had virtually no effect on BPE (data not shown).



Representative chromatographic pattern obtained from the fractionation of benign human prostate extract (Pool 2) on a Sephadex G-200 column. Three identifiable peaks are labeled 1-3 and shown in relationship to the molecular weight standards. The shaded areas correspond to the localization of transglutaminase activity. Values represent the mean of duplicate determinations.

Evaluation of Exogenous Immunomodulatory Factors

Evaluation of the effect of DES and leuprolide was made by comparison of the lytic activity of untreated PBMC treated with DES and/or leuprolide for K-562 target cells.

Variable suppression of NK cell lysis was observed with concentrations of DES ranging from 10 to 100 μM . The use of 100 μM as the optimum inhibitory dose was based on evaluation of the lytic reactivity of PBMC treated with DES or leuprolide at concentrations ranging from 0.1 to 1000 μM .

Individual comparative determinations of the percent lysis of K-562 target cells obtained with untreated and PBMC treated with 100 μM of DES and/or leuprolide are presented in Table V. As PBMC from the same donor were evaluated untreated and treated, each donor served as their own control. Owing to the temporal sequence of specimen availability, and variability in the number of PBMC, the effect of DES and leuprolide was evaluated concomitantly on PBMC from 4 donors, and independently on PBMC from 7 and 9 donors, respectively.

As further shown in Table V, PBMC treated with DES exhibited decreases ranging from 71 to 100% of their lytic activity obtained with untreated cells. In the case of leuprolide, decreases, observed with 5 donors ranged from a negligible 1 and 3% to 23%, with 4 donors showing increases, ranging from 7 to 26%.

The possibility that the observed inhibitory effects of DES or leuprolide on NK cell lysis was due to their cytotoxicity was excluded as the viability (assessed by trypan-blue dye exclusion) of PBMC incubated for 18 hrs. in the culture medium with and without either 100 μM of DES or leuprolide, were essentially identical, i.e., 90%+. Similar results were obtained at 24 hrs., the time corresponding to the completion of the NK cell assay.

A summary comparing the effect of untreated and PBMC treated with DES and leuprolide is shown in Table VI. There was a highly significant 82% reduction from the mean percent lysis of 38.3 for untreated PBMC to 7.0 for DES treated PBMC ($P < 0.001$). In contrast,

TABLE V

Effect of leuprolide and diethylstilboestrol (DES) on the lytic activity of natural killer cells

Donor	Diagnosis	Percent lysis of K-562 target cells obtained with peripheral blood mononuclear cells*		
		Untreated	Treated with 100 μM ** of:	
			Leuprolide (% Change)	DES (% Change)
EB	Healthy	24.8	-	3.5 (- 86)
JB	Healthy	51.5	42.2 (- 17)	-
JB	Healthy	52.2	-	9.7 (- 81)
JBa	Healthy	26.6	25.8 (- 3)	0 (-100)
EJ	Healthy	41.9	41.5 (- 1)	10.1 (- 76)
CL	Healthy	47.6	50.8 (+ 7)	-
DMcL	Healthy	30.0	23.6 (- 21)	-
NM	Healthy	37.1	-	10.7 (- 71)
NMi	Healthy	24.8	19.2 (- 23)	-
RK	BPH	42.7	52.0 (+22)	-
PZ	BPH	28.2	35.7 (+26)	5.3 (- 81)
ER	PCa	52.3	63.6 (+22)	9.6 (- 82)
Means \pm SD		38.3 \pm 11.1 $P > 0.8$ ***	39.4 \pm 14.8 (+ 3) $P < 0.001$ ***	7.0 \pm 4.1 (- 82)***
		P < 0.001***		

* Mean from triplicate determinations at an effector: target cell ratio of 50:1.

** Optimum inhibitory dosage as determined from a dose-response curve ranging from 0.1-1000 μM .

*** Significance (P) of percent change in lysis of untreated peripheral blood mononuclear cells (PBMC) vs. PBMC treated: with Leuprolide or DES, and of PBMC treated with Leuprolide vs. DES.

TABLE VI

Summary of the effect of leuprolide vs. diethylstilboestrol (DES) on the lytic activity of natural killer cells

Peripheral blood mononuclear cells	Percent lysis of K-562 target cells (Mean \pm SD)*	Percent change in lysis from untreated	Significance (P) of percent change in lysis from untreated
Untreated	38.3 \pm 11.1 (12)**	—	—
Treated with 100 μ M***			
Leuprolide	39.4 \pm 14.8 (9)	+ 3	>0.8
DES	7.0 \pm 4.1 (7)	-82	<0.001

* Determined from triplicate determinations on each donor at an effector: target cell ratio of 50:1

**Number of donors evaluated.

***Optimum inhibitory dosage as determined from a dose-response curve ranging from 0.1-1000 μ M.

PBMC treated with leuprolide exhibiting a mean percent lysis of 39.4, for a negligible 3% increase, vs. 38.3 percent for untreated PBMC, showed no significant change in lysis ($P > 0.8$).

Comparable significant differences in the respective effects of DES and leuprolide on NK cell lysis were observed with adherent cell (monocyte) depleted PBMC.

DISCUSSION

In following with the presentation of the "Results", it appears most suitable to conclude initially with a discussion according to the endogenous and exogenous categories of the IM of SeP1 and BPE and DES and leuprolide considered. This will be followed by a summation of the overall suggested relevancy of these IMF to tumour-host responsiveness.

Endogenous Immunomodulatory Factors

This study has demonstrated the IM properties of SeP1 and BPE on the lytic activity of NK cells. In doing so, the results obtained with SeP1 confirm and extend our initial observations of IS of TAI in PCa (Ablyn et al., 1980), and observations of others of the IS activity of SeP1 on a range of immune responses (James & Hargrave, 1984). The observations of IS with BPE extend the range of origin of endogenous IMF to include the prostate gland.

As noted, the IS effect of SeP1 on NK cells and other parameters of immunity has been reported by others. It is, however, significant to note, that with possible exception of the present observations and previous studies of TAI (Ablyn et al., 1980), such IS has been accomplished by simultaneous treatment (incubation) of the effector cell with SeP1 without washing, rather than by pretreatment (preincubation) and

washing of the effector cell prior to use. Therefore, the question of the occurrence of IS as a consequence of merely a physical phenomenon vs. that of an immunologic nature in studies employing simultaneous treatment remains. Paradoxically, we observed (Bartkus et al., 1985), pending the sperm quality of the source of SeP1, stimulation, rather than suppression, of NK cell activity with pretreatment of PBMC with pools of SeP1 prepared from donors with good (normal) sperm quality. This latter observation suggests the sperm quality, and possibly fertility status of the donor source of SeP1, may be relevant to SeP1 IS.

In addition to the comments on SeP1 IS, brief mention should be made of the importance of distinguishing "true" IMF in SeP1 and BPE responsible for their respective IS, from that of the lymphocytotoxic effect of 'acrolein'. 'Acrolein' is formed from the oxidation of SeP1 and prostate polyamines by amine oxidase (Alarcon et al., 1961), present in the plasma or serum of cattle, sheep and other ruminant animals (Kappeler-Adler, 1970), used to supplement tissue culture media, and also in mid-term pregnancy sera (Gaugas & Curzen, 1978). The distinction of this artefactual IS in our studies was made by pretreatment of SeP1 and BPE with the amine oxidase inhibitor-hydroxylamine (Labib & Tomasi, 1981).

Of parenthetical note in reference to amine oxidase, increased concentrations of SeP1 diamine oxidase have been found in association with low sperm density (Janne et al., 1973). This association of sperm quality and diamine oxidase may provide an explanation for our observations of increased IS with SeP1 pools from poor (abnormal) vs. good (normal) sperm quality donors.

Studies directed toward the characterization and identification of the IMF in SeP1 and BPE have provided preliminary data suggestive of the participation of high M_r (100 Kd) and low M_r (≤ 5 Kd) factors. The high M_r factor in SeP1 and BPE appears to be associated, in part with TGase activity. In SeP1, a reduction in the level of IS on dialysis or gel filtration and treatment with the cyclooxygenase inhibitor indomethacin is suggestive of the association of the low M_r factor with prostaglandins. In BPE, dialysis or gel filtration had a greater effect on its IS activity, while treatment with indomethacin had none.

The identification of TGase activity in SeP1, BPE and PCaE was made on the basis of its characteristic catalytic properties (specificity, calcium and thrombin dependency and inhibition by iodoacetamide and anti-FXIII α (BPE only).

The localization of the principal area of TGase activity in SeP1 and BPE by column chromatography is in accord with the M_r for TGase of the guinea pig anterior prostate (Wing et al., 1974), and that identified by Chung (1975) in extract of human prostatic tissue, and by Bures et al., (1980) in extracts of cell cultures of dog and human prostate. Similar to the studies of Bures et al., (1980): i) PCaE possessed significantly higher levels of TGase activity than BPE and ii) evaluation of the resuspended sediment (pellet) and sediment supernatant following further extraction disclosed additional TGase activity in relationship to the cell type (Ablin et al., unpublished observations).

It is emphasized that the present studies of SeP1 and prostate TGase are yet of a preliminary nature. Pending these further studies, including optimization of the assays for SeP1 and prostate TGase, initial results suggest the presence of two types of male accessory sexual gland-associated TGase. One similar, and possibly identical to plasma FXIII, this being of the prostate type. The second as found in SeP1, possibly being a tissue (intracellular), if not, new type of TGase.

The identification of TGase and its presumptively demonstrated association with the IS effect of SeP1 and BPE is particularly significant in view of the implications of TGase in several cellular phenomena (Fesus, et al., 1983). As to the role of TGase in SeP1, several hypotheses have been made by Ablin & Gonder (1985b). In addition to these, TGase may be involved in the crosslinking of fibrin in the tumour stroma.

In this way a: i) crosslinked fibrin network resistant to fibrinolysis is formed between and around tumour cells, that as a barrier may inhibit mechanisms of tumour-host defense, alternately ii) activated FXIII, as a TGase, may covalently attach numerous host proteins to the membrane of tumour cells, thereby covering up the "non-selfness" of tumour. The result of which may be increased immune resistance to the tumour (Adany et al., 1987), e. g., attachment of fibrinogen to the membrane of YPC-1 plasmacytoma cells by tissue TGase inhibited cell-mediated cytotoxicity (Hunyadi et al., 1981).

In consideration of the suggestion that fibrin deposition may inhibit mechanisms of tumour-host defense, Gunji et al., (1987), have recently demonstrated abrogation of the cytotoxic effect of NK or lymphokine activated killer cells in the presence of platelet poor plasma.

In reference to other possibly studies of endogenous urogenital IMF, human prostatic fluid (PF) and extracts of human BPH and dog prostate inhibited: i) phagocytosis and ii) O_2 -consumption and hexomonophosphate activities of granulocytes and macrophages (Stankova et al., 1976; Chvapil et al., 1977). The inhibitory factor was associated with the low M_r (< 5 Kd) fraction of PF and attributed to zinc (Stankova et al., 1976; Chvapil et al., 1977).

It is suggested that observations of endogenous IMF in SeP1 and BPE contribute further toward understanding the: i) predilection for the development of tumours of the prostate compared to their rare occurrence in other male accessory sexual glands; ii) failure of the PCa patient to develop a substantial clinical response to his malignancy in the early stages of disease and iii) inordinate number of patients found on autopsy to have latent PCa. As considered elsewhere by Ablin & Gonder (1985), IS by SeP1 may also be relevant to the poor immunological responsiveness to urogenital infections in the male, including the possible predisposition to HIV infection, wherein it may be a co-factor to the acquired immunodeficiency syndrome (Ablin, 1983a, b; Ablin, 1985b; Ablin & Gonder, 1985b).

On the basis of the present observations and suggested role of the IMF of SeP1 and BPE, it is thought that in addition to the importance of, principally PF and SeP1 as modulators of prostatic function and as a medium for the transport of spermatozoa in reproduction, alterations in their composition, reflected in part by their IS

activity, may serve as indicators, i. e., biological markers, of pathological changes in the prostate. For example, with increases in polyamines and TGase in malignant tissues, the formation of TGase mediated protein-polyamine conjugates might be significant to malignant transformation. In this regard, it perhaps should be emphasized that while IS may not directly induce malignancy, it may compromise the host's ability to control activated tumour cells. As such, IS may be considered as permitting the growth of tumour cells, rather than, perhaps their actual induction. Therefore, it is permissive.

Exogenous Immunomodulatory Factors

Various forms of therapy have significant effects on immune function. In an already possibly immunocompromised cancer patient, these effects may become even more prominent.

The results of the present study have demonstrated the *in vitro* IS effect of DES on the lytic activity of NK cells, within therapeutic levels (Ablin et al., 1979). In confirming the recent report by Kalland & Campbell (1984), and earlier studies of reduced NK cell activity of PBMC from PCa patients receiving DES-P (Kalland & Haukaas, 1981; Onsrud & Sander, 1982), these observations provide; i) further evidence of its IS activity and ii) that leuprolide in addition to its fewer noted clinical side effects, compared to DES (The Leuprolide Study Group, 1984), is also significantly less IS (at least in terms of its effect on NK cell activity) for the K-562 target cell.

Pharmacological, i. e., therapeutic, levels of oestrogens possess a wide range of effects on parameters of immune responsiveness (Grosman, 1985). Oestrogen induced IS may occur, among other possibilities, by a *direct* effect of oestrogen on select populations of the immune micromilieu, e. g., macrophages (Nicol et al.; 1964), OKT8+ PBMC bearing oestrogen receptors (Cohen et al., 1983), and/or their soluble products, e. g., interleukin-2 (Henrikson and Frey, 1982; Pung et al., 1985), prostaglandins (Jondal et al., 1981; Degen et al., 1982), or *indirectly* by increasing the secretion of pituitary prolactin (Chen et al., 1976) and its interaction with prolactin receptors on PBMC (Russell, et al., 1984), or by suppressing secretion of endogenous androgen to support the immune system (Farnsworth, 1981).

Studies in progress are directed toward extension of the present observations and possible delineation of the mechanism(s) of DES-

induced IS. In this regard, it is of interest to note, in accord with the preliminary observations of Djeu et al., (1982), that DES also suppresses the NK cell activity of adherent cell-depleted PBMC for K-562, as well as Molt-4, cell lines (Ablin, unpublished observations).

Significant pharmacologically related IS of NK cell activity by DES and DES-P supports the earlier suggestion that the palliative effects of such therapy may be compromised by a reduction in the patient's immune surveillance to tumour, or equally important, by a decreased capacity to cope with infectious agents (Ablin et al., 1974).

Pending evaluation of the effect of leuprolide on the activity of NK cells for other target cells and other aspects of tumour-host responsiveness, it may prove to be a favorable alternate to DES, not only in view of its fewer noted clinical side effects, but because of its suggested absence of deleterious effects to the immune system. Maintenance of tumour-host equilibrium, and some degree of immunocompetency, in the presence of effective therapy with leuprolide, permitting the further option of adjuvant immunotherapy (Ablin, 1981b), possibly contraindicated by DES-induced IS, may prove most beneficial to achieving more effective therapy in PCa.

The importance of the effects of oestrogen are reflected not only by immunologic considerations, but also by their ability to induce cytogenetic effects, i. e., aneuploidy in lymphocytes (Hill & Wolf, 1982). Aneuploidy represents significant genetic aberrations that may have direct or indirect effects on cellular differentiation (Barrett, 1981).

In addition to the significance of NK cells in immune surveillance against cancer, and in the control of metastases, accumulating evidence suggests NK cells may play an IM role in haemato- and lymphopoiesis (Brieva et al., 1984), and thus, in the end stages of humoral-mediated immunity. It thus becomes obvious that significant alterations in NK cell activity, whether induced by endogenous or exogenous IMF, may have deleterious sequelae in the clinical management of patients with tumours of the prostate.

PCa patients have a high incidence of metastases on initial diagnosis which contribute significantly to the morbidity and mortality of this disease. It is therefore critical that the therapeutic regimen selected maintain homeostasis of the tumour-host relationship and provide

effective control of metastases, least they become exacerbated by the therapy.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the technical assistance of John M. Bartkus (SUNY at Stony Brook, Stony Brook, NY) and the counsel of Doctor Laszlo Muszbek (University School of Medicine, Debrecen, Hungary).

REFERENCES

- ABLIN, R.J., 1977. Immunobiology of the Prostate, pp. 33-98. In Tannenbaum, M. (Ed.): *Urologic Pathology -The Prostate*, Lea and Febiger, Philadelphia.
- ABLIN, R.J., 1981a. Modulatory effects of estrogen on immunologic responsiveness. III. Effect of hormonal versus nonhormonal therapy on tumor-associated immunity in prostatic cancer. *Amer. J. Reprod. Immunol.*, 1 :206-209.
- ABLIN, R.J., 1981b. Adjuvant Immunotherapy for Prostatic Cancer, pp. 251-283. In Ablin, R.J. (Ed.): *Prostatic Cancer*. Marcel Dekker, Inc., New York.
- ABLIN, R.J., 1983a. Acquired immune deficiency syndrome: Toward a possible explanation of its etiology. *IRCS Med. Sci.*, 11 :766-767.
- ABLIN, R.J., 1983b. Immunological Aspects of Benign Prostatic Hypertrophy, pp. 73-98. In Hinman, Jr., F. (Ed.): *Benign Prostatic Hypertrophy*. Springer-Verlag, New York.
- ABLIN, R.J., 1985a. Human prostate antigens: recapitulation and clinical relevance *IRCS Med. Sci.*, 13 :1042-1045.
- ABLIN, R.J., 1985b. Transglutaminase: Co-factor in aetiology of AIDS? (Letter). *Lancet*, 1 :813-814.
- ABLIN, R.J.; BARTKUS, J.M.; GONDER, M.J. & POLGAR, J., 1987. Factors Contributing to Suppression of Tumor-Host Responsiveness, pp. 279-299. In Cimino, F., Birkmayer, C.D., Pimentel, F., Kalvins, J.V. & Salvatore, F. (Eds.): *Proceeding of the 3rd International Conference on Human Tumour Markers - Biology and Clinical Applications*. Walter de Gruyter, Berlin.
- ABLIN, R.J. & BHATTI, R.A., 1981. Tumor-Associated Immunity in Prostatic Cancer pp. 183-204. In Ablin, R.J. (Ed.): *Prostatic Cancer*. Marcel Dekker, Inc., New York.
- ABLIN, R.J.; BHATTI, R.A.; BUSH, I.M. & GUINAN, P.D., 1980. Immunosuppression of cell- and serum-mediated tumour-associated immunity in prostatic cancer by human seminal plasma. *Europ. J. Cancer*, 16 :775-780.
- ABLIN, R.J.; BHATTI, R.A.; GUINAN, P.D. & KHIN, W., 1979. Modulatory effects of oestrogen on immunologic responsiveness. II. Suppression of tumour-associated immunity in patients with prostatic cancer. *Clin. Exp. Immunol.*, 38 :83-91.
- ABLIN, R.J.; BRUNS, G.R.; AL SHEIK, H.; GUINAN, P.D. & BUSH, I.M., 1976. Hormonal therapy and alteration of lymphocyte proliferation. *J. Lab. Clin. Med.*, 87 :227-231.
- ABLIN, R.J.; BRUNS, G.R.; GUINAN, P. & BUSH, I.M., 1974. The effect of estrogen on the incorporation of ³H-thymidine by PHA-stimulated human peripheral blood lymphocytes. *J. Immunol.*, 113 :705-707.
- ABLIN, R.J. & GONDER, M.J., 1985a. Immunological Aspects of Benign and Malignant Tumors of the Prostate, pp. 185-198. In Keller, P.J. (Ed.): *Contributions to Gynecology and Obstetrics*, Vol. 14, S. Karger, Basel
- ABLIN, R.J. & GONDER, M.J., 1985b. Male Accessory Sexual Glands Secretions and Their Antithetical Role in Immunosurveillance, pp. 271-276. In Peeters, H. (Ed.): *Protides of the Biological Fluids*, Vol. 32. Pergamon Press, Ltd., Oxford.
- ADANY, R.; NEMES, Z. & MUSZBEK, L., 1987. Characterization of Factor XIII containing-macrophages in lymph nodes with Hodgkin's disease. *Brit. J. Cancer*, 55 :421-426.
- ALARCON, R.A.; FOLEY, G.E. & MODEST, F.J., 1961. Effects of spermine on mammalian cells. *Arch. Biochem. Biophys.*, 94 :540-541.
- BARRETT, J.C., 1981. Cell transformation, mutation and cancer. *Gann*, 27 :195-206.
- BARTKUS, J.M.; ABLIN, R.J.; REDA, A.; KULIKAUSKAS, V. & GONDER, M.J., 1985. Immunobiological studies of human seminal plasma (Abstract). *Amer. J. Reprod. Immunol. Microbiol.*, 7 :139-140.
- BIRKHOFF, J.D., 1983. Natural History of Benign Prostatic Hypertrophy, pp. 59. In Hinman, Jr., F. (Ed.): *Benign Prostatic Hypertrophy*. Springer-Verlag, New York.
- BRADFORD, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, 72 :248-254.
- BRIEVA, J.A.; TARGAN, S. & STEVENS, R.J., 1984. NK and T cell subsets regulate antibody production by human *in vivo* antigen-induced lymphoblastoid B cells. *J. Immunol.*, 132 :611-615.
- BRONSON, P.; ABLIN, R.J. & SOANFS, W.A., 1970. Evaluation of the freeze-thaw alteration of rabbit mare urogenital tissue antigens by double diffusion gel precipitation. *Cryobiol.*, 6 :401-403.
- BRUNNER, K.T.; MAUEL J.; CEROTTINI, J.-C. & CHAPUIS, B., 1968. Quantitative assay of the lytic action of immune lymphoid cells on ⁵¹Cr-labelled allogeneic target cells *in vitro*: Inhibition by iso-antibody and by drugs. *Immunol.*, 14 :181-196.
- BURES, M.; GOLDSMITH, L.A. & STONE, K.R., 1980. Transglutaminase activity of cultured human prostatic epithelium. *Invest. Urol.*, 17 :298-301.
- CHEN, C.L.; PATTISON, M.L.; ENGLEKING, L.R. & GRONWALL, R.R., 1976. Studies on metabolism and effects of estrogen on pituitary prolactin and LH secretion. *J. Toxicol. Environ. Hlth.*, 1 :641-655.
- CHUNG, S.I., 1975. Multiple Molecular Forms of Transglutaminases in Human and Guinea Pig, pp. 259-274. In Markert, C.L. (Ed.): *Isoenzymes*. Vol. 1, Academic Press, New York.
- CHVAPIL, M.; STANKOVA, L.; BERNHARD, D.S.; ZUKOSKI, C.F. & DRACH, G.W., 1977. Effect of prostatic fluid and its fraction on some functions of peritoneal macrophages. *Invest. Urol.*, 15 :173-179.
- COHEN, J.H.M.; DANIEL, L.; CORDIER, G.; SAEZ, S. & REVILLARD, J.-P., 1983. Sex steroid receptors in peripheral T cells. Absence of androgen

- receptors and restriction of estrogen receptors to OKT8-positive cells. *J. Immunol.*, *131* :2767-2771.
- DEGEN, G.H.; ELING, T.E. & McLACHLAN, J.A., 1982. Oxidative metabolism of diethylstilbestrol by prostaglandin synthetase. *Cancer Res.*, *42* :919-923.
- DJEU, J.Y.; STOCKS, N. & RAMSEY, K.M., 1982. Inhibition of human natural killer (NK) cell activity by immunopharmacological agents and time-dependent reversal of inhibition by interferon (IFN) (Abstract). *Immunobiol.*, *163* :213-214.
- FARNSWORTH, W.E., 1981. Stimuli, Receptors, and Effectors of Prostatic Function, pp. 105-119. In Ablin, R.J. (Ed.): *Prostatic Cancer*. Marcel Dekker, Inc., New York.
- FESUS, L.; HORVATH, A. & HARSFALVI, J., 1983. Interaction between tissue transglutaminase and phospholipid vesicles. *FEBS Letters*, *155* :1-5.
- GAUGAS, J.M. & CURZEN, P., 1978. Polyamine interaction with pregnancy serum in suppression of lymphocyte transformation. *Lancet*, *1* :18-20.
- GRIFFITHS, J., 1982. Clinical Applications of Fluoroimmunoassay: An Evaluation in the Diagnosis of Early Prostate Adenocarcinoma, pp. 1241-1249. In Kaiser, E., Gabl, M., Muller, M. & Bayer, M. (Eds.): *IX International Congress of Clinical Chemistry*. Walter de Gruyter & Co., Berlin.
- GROSSMAN, C.J., 1985. Interactions between the gonadal steroids and the immune system. *Science*, *227* :257-261.
- GUNJI, Y.; LEWIS, J.; HERBERMAN, R. & GORELIK, E., 1987. Mechanisms of the antimetastatic action of anticoagulants. II. Fibrin coagulation protects tumor cells from destruction by NK or LAK cells (Abstract). *Proc. Amer. Assoc. Cancer Res.*, *28* :285.
- HAUKAAS, S., HOISAETER, P.A. & KALLAND, T., 1982. *In vitro* and *in vivo* effects of diethylstilbestrol and estramustine phosphate (Estracyt) on the mitogen responsiveness of human peripheral blood lymphocytes. *The Prostate*, *3* :405-414.
- HENRIKSEN, O. & FREY, J.R., 1982. Control of the expression of interleukin 2 activity. *Cell. Immunol.*, *73* :106-114.
- HERBERMAN, R.B., 1982. *NK cells and Other Natural Effector Cells*. Academic Press, Inc., New York.
- HILL, A. & WOLFF, S., 1982. Increased induction of sister chromatid exchange by diethylstilbestrol in lymphocytes from pregnant and premenopausal women *Cancer Res.*, *42* :893-896.
- HUNYADI, J.; SZEGEDI, G.; SZABO, T.; AHMED, A. & LAKI, K., 1981. Increased cytotoxic sensitivity of YPC-1 tumor cells from mice treated with nitrosureas. *Cancer Res.*, *41* :1677-1681.
- JAMES, K. & HARGREAVE, T.B., 1984. Immunosuppression by seminal plasma and its possible clinical significance. *Immunol. Today*, *5* :357-363.
- JANNE, J.; HOLTTA, E.; HAARANEN, P. & ELFVING, K., 1973. Polyamines and polyamine-metabolizing enzyme in human semen. *Clin. Chim. Acta*, *48* :393-401.
- JONDAL, M.; MERRILL, J. & ULLBERG, M., 1981. Monocyte-induced human natural killer cell suppression followed by increased cytotoxic activity during short-term *in vitro* culture in autologous serum. *Scand. J. Immunol.*, *14* :555-563.
- KALLAND, T. & CAMPBELL, T., 1984. Effects of diethylstilbestrol on human natural killer cells *in vitro*. *Immunopharmacology*, *8* :19-25.
- KALLAND, T. & HAUKAAS, S., 1981. Effect of treatment with diethylstilbestrol-polyestradiol phosphate or estramustine phosphate (Estracyt) on natural killer cell activity in patients with prostatic cancer. *Invest. Urol.* *18* :437-439.
- KAPPELLER-ADLER, R., 1970. *Amine Oxidases and Methods of Their Study*. Wiley-Interscience, New York, p. 26.
- LABIB, R.S. & TOMASI, JR., T.B., 1981. Enzymatic oxidation of polyamines. Relationship to immunosuppressive properties. *Eur. J. Immunol.*, *11* :266-269.
- LORAND, L.; CAMPBELL-WILKES, L.K. & COOPERSTEIN, L., 1972. A filter paper assay for transamidating enzymes using radioactive amine substrates. *Anal. Biochem.*, *50* :623-631.
- NICOL, T., BILBEY, D.; CHARLES, L.; CORDINGGLEI, J. & VERNON-ROBERTS, B., 1964. Oestrogen: The natural stimulant of body defense. *J. Endocrin.*, *30* :277-291.
- ONSRUD, M. & SANDER, S., 1982. Influence of *in vivo* diethylstilbesterol phosphate on some human blood lymphocyte sub-populations. *Acta Path. Microbiol. Immunol. Scand., Section C*, *90* :271-276.
- PUNG, O.J.; TUCKER, A.N.; VORE, S.J. & LUSTER, M.I., 1985. Influence of estrogen on host resistance: Increased susceptibility of mice to *Listeria monocytogenes* correlates with depressed production of interleukin 2. *Infect. Immunity*, *50* :91-96.
- RUBENSTEIN, M.; SHAW, M.W.; McKIEL, C.F.; STOBNIKI, M.; ABLIN, R.J. & GUINAN, P.D., 1985. The effect of hormone therapy on peripheral blood leukocytic subset distribution in Stage D prostatic cancer patients. *Urol. Res.*, *13* :67-71.
- RUSSELL, D.H.; MATRISTIAN, L.; KIBLER, R.; LARSON, D.F.; POULIS, B. & MAGUN, B.E., 1984. Prolactin receptors on human lymphocytes and modulation by cyclosporine. *Biochem Biophys. Res. Comm.*, *121* :899-906.
- STANKOVA, L.; DRACH, G.W.; HICKS, T.; ZUKOSKI, C.F. & CHVAPIL, M., 1976. Regulation of some functions of granulocytes by zinc of the prostatic fluid and prostate tissue. *J. Lab. Clin. Med.*, *88* :640-648.
- THE LEUPROLIDE STUDY GROUP, 1984. Leuprolide versus diethylstilbestrol for metastatic prostate cancer. *N. Eng. J. Med.*, *311* :1281-1286.
- TULINIUS, H., 1982. Latent Malignancies, pp. 129-137. In Grundmann, E. (Ed.): *Cancer Epidemiology, Cancer Campaign, Vol. 6*. Gustav Fischer Verlag, Stuttgart, New York.
- WING, D.; CURTIS, C.G.; LORAND, L. & WILLIAMS -ASHMAN, H.G., 1974. Isolation of a transglutaminase from the coagulating gland of the guinea pig prostate (Abstract). *Fed. Proc.*, *33* :290.