The increase in intracellular pH during mitogen stimulation in fibroblasts results from the activation of the Na^+/H^+ exchange system[8–11]. Since DMSO-induction of Friend-erythroleukemia cells results in a decrease in Na^+ transport into the cell[20,21], it is possible that reduction in the Na^+/H^+ exchange system is responsible for the decrease in pH after DMSO treatment. This will require further investigation, since the activity of the Na^+/H^+ exchange system is relatively low during normal growth conditions in the exponential phase[22].

Our results also show a brisk decrease in cell water volume after DMSO treatment of F4–6 cells. This occurs within 15 min. With other techniques the decrease in cell volume after DMSO induction was first observed after 8–10 h[23,24]. The quick volume decrease may be indicative of changes in the cell membrane during the very early phase of the induction of differentiation with DMSO.

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Inhibition of sensitized leukocyte's in vitro reactivity by circulating immune complexes in prostate cancer

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Summary. Circulating immune complexes in the sera of patients with confirmed histological diagnosis of carcinoma of the prostate, were found to interfere in the sensitized leukocyte's in vitro reactivity to prostate cancer associated antigen as evaluated by tube leukocyte adherence inhibition assay, thereby suggesting an inhibitory role of such serum factors in host's anti tumor cell mediated immune responses.
Key words. Circulating immune complexes; prostate cancer, inhibition.

The failure of immune responses in tumor bearing hosts to control tumor growth results from the intervention of a series of host factors that diminish the effectiveness of cell mediated immunity. One of such factors are antigen/antibody complexes which are reported to have an adverse effect on cell mediated reactions in humans as well as in experimental tumor systems. In a number of studies circulating immune complexes (CIC) were found either to reduce[1] or abrogate [2–5] the cell mediated cytotoxicity against cultured tumor cells and even interact with the receptors on cells resulting in the release of biologically active mediators which may interfere with cellular functions or the cells' recognition mechanism[6]. The presence of such complexes in patients with cancer of the prostate (CaP), and the alteration of such levels by anti neoplastic therapy have already been reported[7,8]. The present investigations were undertaken to evaluate any in vitro inhibitory effect of prostate cancer CIC on cells' recognition mechanism.

Materials and method. Patients. Blood samples were obtained from 10 patients with confirmed histological diagnosis of adenocarcinoma of the prostate who were receiving various types of therapy at the time of evaluation. Serum samples were stored at −20 °C. In order to evaluate the effect of CIC on cellular functions, leukocytes from 5 control subjects with no clinical signs or symptoms of any malignancy or immunologic disorder were used.

Polyethylene glycol precipitation assay for measuring CIC. Serum circulating immune complexes in the sera of patients with cancer of the prostate were measured as detailed previously[7]. Briefly, diluted serum samples were mixed with a 3.75% solution of polyethylene glycol (MW 6000), and with 0.1 M borate buffer to serve as controls. All tubes were run in

In vitro effect of serum circulating immune complexes on sensitized leukocytes reactivity

Groups	Mean CIC level ± SD (OD 450 × 10^{-3})	LAI response to PcAA (mean % non adherence ± SD)
1	24.1 ± 9.3	57.2 ± 14.9
2	55.5 ± 24.3	33.9 ± 11.3
$p <$	0.05	0.05

duplicate and kept at room temperature for 120 min. Immune complexes were quantitated by measuring the optical density of the samples at 450 nm using a Beckman model 25 spectrophotometer. The results were calculated in the following fashion: (OD450 × 10^3 with PEG) – (OD450 × 10^3 with borate buffer).

Effect of CIC on cells recognition mechanism. In order to evaluate the effect of circulating antigen/antibody complexes found in the sera of patients with cancer of the prostate, on cells' recognition mechanisms, Ficoll Hypaque isolated leukocytes from control subjects were first sensitized by incubation with CaP sera for 30 min. These cells were then washed with MEM before reacting with an ammonium sulfate precipitate of prostate cancer associated antigen (PcAA) prepared from the malignant prostatic tissue. Employing a leukocyte adherence inhibition assay (LAI), in which sensitized leukocytes recall, recognize and react with the specific tumor antigen and lose their glass adherence property, the effect of CIC on these cellular functions was evaluated by measuring their in vitro reactivity to PcAA. The details of LAI, arming technique[9] and preparation of tumor antigen[10] have been described previously.

Results. CIC levels measured in the sera of CaP patients did not correspond to the stage of malignancy, perhaps due to the effect of various types of therapeutic modalities these patients were receiving at the time of their evaluation[7], and were divided into two distinct groups. One with lower levels of CIC and the other with higher levels, i.e. twice as high as group one. The difference in CIC levels between the two groups was statistically significant $p < 0.05$ (table). When control leukocytes were pre-sensitized with sera from patients with lower levels of CIC, their in vitro response to PcAA as judged by LAI, was much higher as compared to the response of leukocytes pre-sensitized with elevated levels of CIC. The difference in leukocytes in vitro reactivity to PcAA, between cells pre-treated with sera containing lower levels of CIC vs those pre-treated with sera containing higher levels of CIC was statistically significant at $p < 0.05$ (table).

Discussion. Interference in a tumor bearing host's cell mediated immunity may be produced by circulating serum factors which interact and even mask (neo)antigens on tumor cells, thus preventing their recognition by sensitized leukocytes, a phenomenon often referred to as 'blocking'. Existence of such factors in prostate cancer has already been reported[11]. On the other hand serum factors from the tumor bearing host may directly interact with the effector cells, thereby inhibiting their reactivity to tumor associated antigen. This type of response has been termed as 'inhibitory'. The data obtained in the present study, where cells from the same control subjects when pretreated with sera from different CaP patients containing various levels of CIC responded so differently to PcAA, demonstrate an in vitro inhibitory effect of circulating antigen/antibody complexes. Such an effect of CIC appears to be directly proportional to the quantity of CIC in the serum, i.e. the higher the levels of CIC, the greater the inhibitory effect as judged by the lower degree of sensitized leukocytes reactivity to tumor associated antigen. Whether such complexes have a similar inhibitory effect on a host's in vivo anti tumor cell mediated immunity and whether these complexes have any immunomodulatory effect on a host's overall anti tumor responses needs to be investigated. Our preliminary findings in a prostatic tumor model, i.e. Dunning's R3327 MatLyLu tumor, suggest the existence of an in vivo inhibitory effect of CIC on host's cellular responses.

Even if the non antigen specific nature of polyethylene glycol precipitation technique for isolating CIC form CaP patient's sera is recognized, the fact is that these patients (who were not undergoing any type of immunosuppressive therapy which could make them more susceptible to a variety of infections and may result in the formation of complexes unrelated to tumor), had different levels of CIC. And when leukocytes from control subjects were pre-sensitized with these, sera gave different degrees of in vitro response to PcAA. When the level of CIC was higher in the serum, the arming potential was lower hence the leukocyte's reactivity to PcAA, suggesting the existence of an inhibitory role of CIC in a host's tumor rejection attempts.

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