we synthesized m-maleimidobenzoyl derivative of hapten for coupling to sulfhydryl group of the enzyme, resulting in high efficiency of binding to the enzyme without appreciable reduction in enzyme activity. When we compared this enzyme labeled hapten with the conjugate prepared identical to that of hapten-protein conjugation for immunization, our conjugate preparation resulted in increased assay sensitivity (figure 2). Since similar results have been often observed in radioimmunoassay for the preparation of tyrosine or tyramine derivative of haptens as radioiodinated ligants[12], our studies showed for the first time the importance of modification around the bridge for increased EIA assay sensitivity, although it is also important to ensure that the antigenic groups exposed upon the hapten-protein conjugate employed for immunization are available in the enzyme-coupled hapten complex.

A high sensitivitiy observed in our T_4 EIA reported previously may also be due to the similar modification around the bridge in hapten-enzyme complex formation[3].

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Suppression of cytophilic antibody ('arming' factor) in the sera of patients with prostatic cancer by human seminal plasma

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Summary. The 'arming' of normal peripheral blood leukocytes (PBL) by cytophilic antibody in the sera of prostatic cancer patients is suppressed by pretreatment of PBL with normal human seminal plasma (HuSPl). Suppression of cytophilic antibody by HuSPl extends the spectrum of immunologic reactions on which SPl has an immunosuppressive effect and may provide further insight into the possible role of SPl in the natural history of prostatic cancer.

The immunosuppressive properties of the hormonal and/or secretory milieu or tumour-elaborated factors (in the case of carcinoma) of the prostate have been suggested[2] as 1 explanation for the hypothesized immunologic privilegedness of the prostate[3]. In an initial study of the role of one of these factors as contributory to the privileged status of the prostate, normal human seminal plasma (HuSPl) has been observed to suppress tumour-associated immunity in patients with prostatic cancer[4].

To possibly further elucidate the immunosuppressive effects of HuSPl and its role in tumour-host responsiveness, the effect of HuSPl on a stage-related and disease-specific activating or 'arming' factor (cytophilic antibody) in the sera of prostatic cancer patients[5,6] has been evaluated.

Materials and methods. Peripheral blood leukocytes (PBL) were obtained from 8 normal adult individuals, ranging in age from 26–67 years, by Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) centrifugation using a modification[7] of the method of Boyum[8]. PBL at a concentration of 1×10^7 cells/ml in RPMI 1640 medium (Grand Island Biological Company, Grand Island, New York), containing 100 IU penicillin g/ml and 100 µg streptomycin/ml, untreated and treated with 280 µg/ml of pooled normal HuSPl[4] were 'armed' by incubation at 37 °C for 50 min with 1:2 dilutions of serum from each of 7 patients with localized (stage A) and metastatic (stage D) prostatic cancer[6]. After incubation, cells were washed twice in RPMI 1640 medium and viability assessed by trypan-blue dye exclusion.

Employing a modification[7] of the tube leukocyte adherence inhibition method[9], untreated and 'armed' PBL and PBL treated with HuSPl and 'armed' were reacted with 3M KCl-$(NH_4)_2SO_4$ extracts of allogeneic malignant prostate[7]; and the number of nonadherent cells counted in quadruplicate using a Standard Neubauer haemocytometer.

Delineation of the significance of the effect of HuSPl on the 'arming' of normal PBL and their reactivity with malignant prostate compared with the reactivity obtained with untreated and 'armed' normal PBL was determined by the paired t-test.

Effect of human seminal plasma (HuSPl) on 'arming' of normal peripheral blood leukocytes with serum from patients with localized and metastatic prostatic cancer

Serum from stage[a]	Mean ± SE % nonadherent cells obtained with malignant prostate and normal leukocytes[b]		Significance[c]
	Untreated and 'armed'	Treated with HuSPl and 'armed'	
A (Localized)	24.1 ± 2.7	12.8 ± 1.7	$p < 0.05$
D (Metastatic)	13.3 ± 2.0	7.5 ± 2.0	$p < 0.05$
Significance[c]	$p < 0.05$	$p > 0.05$	

[a] Serum from 7 patients with localized prostatic cancer (stage A) and 7 patients with metastatic prostatic cancer (stage D); [b] from 8 normal adult volunteers; [c] paired 't'-test.

Results and comment. The effect of HuSPl on the 'arming' of normal PBL with serum from patients with localized (stage A) and metastatic (stage D) prostatic cancer and their degree of reactivity with malignant prostate is shown in the table. Comparison of the significance of the differences in responsiveness expressed as the mean ± SE percent of nonadherent cells obtained with untreated and 'armed' PBL and PBL treated with HuSPl prior to 'arming' and malignant prostate indicated significant differences ($p < 0.05$).

As further shown in the table, and in keeping with previous studies of 'arming' of normal PBL, the reactivity of untreated PBL 'armed' with serum from patients with localized disease and malignant prostate was significantly ($p < 0.05$) greater than that obtained when the same PBL were 'armed' with serum from patients with metastatic disease and reacted with malignant prostate. However, while a difference between the reactivity of PBL treated with HuSPl and 'armed' with serum from patients with localized and metastatic disease and malignant prostate was observed, this difference was no longer significant ($p > 0.05$).

When normal PBL were 'armed' with serum from patients with localized or metastatic disease prior to treatment with HuSPl and reacted with malignant prostate, no reduction in responsiveness was noted.

The present observations provide further evidence of the suppressive effect of HuSPl on tumour-associated immunity in patients with prostatic cancer[4] and extend the type of immunologic reactions on which SPl has an immunosuppressive effect.

The availability and binding of cytophilic antibody to monocyte receptors may be critical to 'arming' and possibly in the presentation of antigen to reactive cells requisite for the induction of various immune responses. Suppression of these functions by HuSPl may provide further insight into the role of SPl and the natural history of prostatic cancer[4].

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Immunological cross reaction between some cattle and sheep allotypic markers

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Summary. The cattle allotypic marker Mca_1 cross-reacted with sheep allotypes $A_{1,2}$ and A_2, removing anti A_2 antibodies from sheep alloantiserum. It would appear that the cross-reaction is due to a resemblance between such antigens closer than that suggested by the role that Mannose plays in determining their serological activity.

Analysis of antigenic properties of serum proteins from various species shows that the patterns of cross-reaction can be correlated with the evolutional relationships of the animals from which such proteins derived[2]. Since the allotypes (serum antigens identifiable by alloimmunization and Mendelian inherited) can be regarded as genetic markers, it seemed appropriate to utilize such molecules and related alloantisera in order to monitor, by the analysis of the cross-reactions, the phylogenetic relationships of serum proteins within a cluster of closely related species such as that of ruminants; the present paper deals with a part of this general project[3-8].

Mca_1 is a cattle allotypic form, carried on a high molecular weight serum glycoprotein, eluting in the 1st peak on sephadex G-200[9], whose antigen activity is determined by mannosyl residues, localized in the prosthetic portion of the molecule[3]; cattle sera showing this antigen activity are called $Mca_1(+)$.

$A_{1,2}$ and A_2 are 2 sheep allotypic forms, carried on a low molecular weight serum glycoprotein, eluting in the 3rd peak on sephadex G-200, whose antigen activity is likewise determined by Mannose[7]; sheep sera showing these antigen activities are called $A_1(+)$ and $A_2(+)$ respectively.

The immunodominat role that Mannose plays in determining both the Mca_1 cattle and the $A_{1,2}$ and A_2 sheep antigen specificities suggested that these antigens could cross-react towards the antiserum directed against one of them. Thus, in view of the general project mentioned, it seemed interesting to investigate whether such a cross-reaction occurred and whether a further resemblance could be established between the cattle and sheep glycoproteins carrying the allotypic markers.

Materials and methods. Double diffusion (DD) was performed as described by Iannelli[5]. Absorption tests were performed by incubating cattle Mca_1 serum and sheep antiA_1, antiA_2 alloantiserum in the ratio v/v = 1/5, as already described[7].

Cattle alloantiserum antiMca_1 dilutions

Inhibitors	1/50	1/100	1/200	1/400
$Mca_1(+)$	+	−	−	−
$A_2(+)$	++	+	−	−
$A_1(+)$	++	++	+	−
$Mca_2(+)$	++	++	++	+
Buffer	++	++	++	+

Agglutination inhibition activity by both cattle-Mca_1 (+) and Mca_2 (+)- and sheep-A_1 (+) and A_2 (+)- sera towards the reaction: SRC-Mca_1+ antiMca_1. Experimental conditions: 0.025 ml of antiserum antiMca_1, at different dilutions, plus an equal volume of each testing serum were incubated for 30 min at r.t. and then 0.025 ml of sheep red cells coated with Mca_1 antigen were added. ++ Indicated strong agglutination reaction (0% inhibition); + indicated weak agglutination reaction; - indicated no agglutination reaction (100% inhibition).