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.

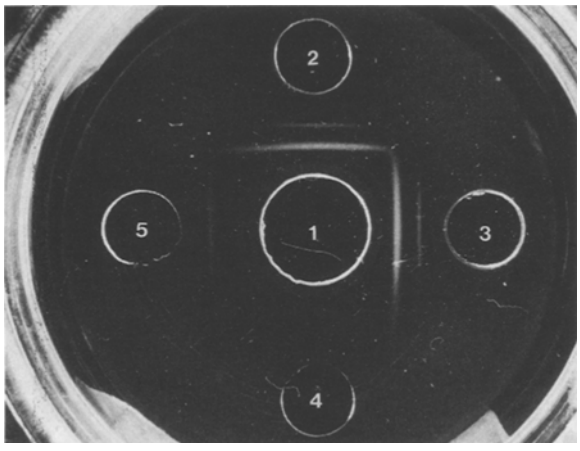
The immunodominat role that Mannose plays in determining both the Mca_1 cattle and the $A_{1,2}$ and A_2 sheep antigen specificities suggested that these antigens could cross-react towards the antiserum directed against one of them. Thus, in view of the general project mentioned, it seemed interesting 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).



Double diffusion pattern of A_2 and Mca_1 antigen preparations denatured and reduced by SDS and 2 mercaptoethanol. Well 1: sheep alloantiserum $antiA_1$, $antiA_2$; well 2: Mca_1 antigen preparation after incubation at 100 °C for 2 min with a buffer of 0.01 M sodium phosphate, pH 7.0, containing SDS and 2 mercaptoethanol (both 1%); well 3: A_2 antigen preparation treated as described for Mca_1; wells 4 and 5: Mca_1 and A_2 antigen preparations treated with a buffer devoid of SDS and 2 mercaptoethanol.

Passive hemagglutination inhibition test (PHI) was performed in Microtiter trays by incubating 0.025 ml of the antiserum $antiMca_1$ at 4 different dilutions (1/50, 1/100, 1/200, 1/400) with an equal volume of such sera, whose inhibiting power was to be checked; after 30 min incubation time at room temperature, 0.025 ml of sheep red cells coated with Mca_1 by concanavalin A[3] were added to each cup. Trays were read after 1 h and again after 2 h from the 1st reading.

Gel filtration on sephadex G-200 was performed according to Iannelli[4].

Denaturation and reduction by SDS and 2 mercaptoethanol[10] were performed by incubating 0.1 ml of Mca_1 and A_2 antigen preparations (obtained by gel filtration on sephadex G-200 from homozygous cows Mca_1/Mca_1 and homozygous sheep A_2/A_2) with 0.9 ml of 0.01 sodium phosphate buffer, pH 7.0, containing 1% SDS and 1% mercaptoethanol at 100 °C. After 2 min the samples were cooled at room temperature and checked at DD against the antiserum containing the $antiA_2$ antibodies. In the standard reaction, the buffer was devoid of SDS and 2 mercaptoethanol.

All sera and cattle and sheep alloantisera were kindly given by Prof. Iannelli, Institute of Animal Production, University of Naples.

Results. Absorption tests revealed firstly that the cattle glycoprotein Mca_1 could remove the antibodies directed against the antigen specificity A_2 from sheep alloantiserum: in fact, when Mca_1 (+) sera were used as absorbers in these tests, after incubation the supernatant of the sheep alloantiserum $antiA_1$, $antiA_2$ reacted at DD with A_1 (+) but not A_2 (+) sheep sera, thus suggesting that Mca_1 cattle sera had removed the $antiA_2$ sheep alloantibodies. The specificity of the reaction was confirmed by replacing Mca_1 (+) with Mca_2 (+) cattle sera (Mca_2 is the product of an allelic form at the same locus Mca[6], whose antigen activity is specified by glucose[3]; in no case did sheep alloantiserum deplete its activity but, after incubation with Mca_2, the supernatant reacted at DD with both A_1 (+) and A_2 (+) sheep sera, thus suggesting that Mca_2 had removed no antibody population from sheep alloantiserum.

In order to ascertain whether sheep $A_{1,2}$ and A_2 glycoproteins could likewise interact with cattle $antiMCa_1$ alloantibodies, the inhibiting power of the A_1 (+) and A_2 (+) sheep sera towards the reaction Mca_1-$antiMca_1$ was checked by PHI tests; to this purpose, SRC coated with Mca_1 were made to react in Microtiter trays with $antiMca_1$ alloantiserum, properly incubated with the following sera: Mca_1 (+), A_1 (+), A_2 (+), Mca_2 (+). As expected, the first and the last serum inhibited at 100% and 0% respectively; A_1 (+) serum, whose antigen activity is due to $A_{1,2}$ molecules inhibited less than A_2 (+) serum, whose inhibiting power, due to molecules A_2, proved rather similar to that of Mca_1 (+) serum (table).

These results indicated that both cattle and sheep alloantibody populations, $antiMca_1$ and $antiA_2$, were directed against a molecular shape common to Mca_1, $A_{1,2}$, A_2 glycoproteins; it seemed worth investigating whether this common shape is only due to the mannosyl residues (as in the case of the blood group substances A, B, H, Le^a which crossreact with type XIV pneumococcus, for prosthetic determinants[11] or whether the cross-reaction might involve a further and closer resemblance between the antigens. To this purpose, Mca_1 and A_2 antigen preparations, separated from whole sera by gel filtration on sephadex G-200, were denatured and reduced by SDS and mercaptoethanol and the resulting fragments were checked at DD in adjacent wells against the sheep alloantiserum, containing the $antiA_2$ antibodies. After 24 h incubation time, the pattern shown in the figure resulted (wells 2 and 3): 2 precipitin lines appeared for each reaction system and each line coalesced with the adjacent one, indicating 2 identity reactions. It must be observed that in the standard reaction with both Mca_1 and A_2 untreated, only A_2 reacted, while no precipitin line was displayed by Mca_1 (figure, wells 4 and 5); the absence of an evident precipitation Mca_1-$antiA_2$ antibodies in this test could be due to the experimental conditions.

Discussion. The role played by Mannose in determining both the Mca_1 cattle and the $A_{1,2}$ and A_2 sheep antigen specificities accounts for the cross-reaction displayed by these allotypes in the passive agglutination inhibition test, in which the $antiMca_1$ cattle alloantiserum was specifically absorbed not only by the cattle Mca_1 but also by the sheep $A_{1,2}$ and A_2 antigens. However, the finding that a prosthetic determinant is common to 2 glycoproteic allotypes of different species implies that in both species the allotypic polymorphism is sustained by genes mediating the addition of the same glycosil units to the macromolecules displaying the antigen specificities[3,7].

An interesting result was obtained when the cattle Mca_1 and the sheep A_2 reduced fragments were checked at DD against the $antiA_2$ sheep alloantiserum (figure): the antiserum reacted in the same manner with the molecular populations arising from both the cattle and the sheep SDS-mercaptoethanol treated allotypes, clearly displaying an identity reaction between them. Further, the intercept spurring from the untreated A_2 antigen to the Mca_1 reduced fragments were recognized by the sheep alloantiserum as being a part of the sheep A_2 antigen. The whole pattern suggested that 2 common antigen subunits were released from both A_2 and Mca_1 by reduction.

It thus seems that the resemblance between these cattle and sheep allotypic markers is not only due to the mannosyl residues, which are immunodominant in the antigen activity, but that such a resemblance might be much closer and perhaps also involve the proteic portion of these cattle and sheep glycoproteins; if so, Mca_1 (mol.wt 900,000) could be a polymeric form of A_2 (mol.wt smaller than 100,000); likewise, the B_1 allotype, identified by alloimmunization in sheep and recognized to be present also in cattle, showed a different molecular weight in both the species[8].

The next steps in our study will be to discover whether such

antigen subunits common to Mca_1 and A_2 are to be found in the glycoproteic allotypes already identified in other species of ruminants (goat and buffalo) and to carry out their physicochemical analysis, but already the present results are interesting in that they provide meaningful experimental evidence to support the general project (the monitoring of phylogenetic relationships by cross-reacting allotypes) under study.

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Destruction of virus infected cells by neutrophils and complement[1]

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Summary. The paper describes an antibody independent mechanism of cytotoxicity whereby virus infected but not uninfected cells are destroyed by the combined presence of neutrophils and complement.

The quest to understand immunity to viruses by studying in vitro models has revealed a diverse array of mechanisms by which virus infected cells may be destroyed. These mechanisms include antibody and complement lysis[3], T-cell-mediated cytotoxicity[4], antibody dependent cellular cytotoxicity (ADCC)[5], complement facilitated ADCC[6], activated macrophages[7,8], natural killer cells[9,10], and mitogen induced cellular cytotoxicity[11,12]. The present report adds another mechanism, namely complement dependent neutrophil mediated cytotoxicity (CDNC). Since this mechanism is nonimmune, it could represent an important early defense mechanism against virus infections.

Materials and methods. Bovine PMN and macrophages were isolated from the mammary gland and lymphocytes from the peripheral blood by methods described in detail elsewhere[13]. The PMN were 98–99% pure and were contaminated by macrophages. 4 different donor animals were used; 2 of which had antibodies (A and B) to infectious bovine rhinotracheitis virus (IBR) and 2 without (C and D). The methods used to detect antibody were virus neutralization and an antibody dependent cell cytotoxicity assay. Both of these methods were described previously[14]. The rabbit complement source was also checked for anti-IBR antibody. None of the 4 animal donors contained detectable antibodies to herpes simplex virus type 1 (HSV) or vescicular stomatitis virus (VSV).

Cytotoxicity assays. The methods used for performing cytotoxicities have been described previously[15]. Briefly Georgia Bovine Kidney cells (GBK) were infected 20 h prior to assay at a multiplicity of infection of 1 with IBR virus. These cells were labelled with $Na_2{}^{51}CrO_4$ and served as targets. Uninfected GBK cells served as control targets. In some experiments, GBK cells infected with HSV or VSV were used as target cells. Rabbit complement (ICN Pharmaceuticals, Cleveland) was freshly reconstituted in Hank's balanced salt solution and dilutions were made to give final complement concentrations of 1/50, 1/70 and 1/100 in the cytotoxicity assays. Heat inactivated (56 °C 30 min) complement was used in control assays. All assays were run for 8 h at 37 °C and were carried out in quadruplicate in microtiter plates[13]. At the end of the incubation period, 50% of the contents were harvested to compute the percent specific release values according to the following formula.

$$\frac{(\text{PMN}+\text{C})-(\text{C}+\text{medium})}{\text{Triton releasible}-(\text{C}+\text{medium})}\times 100$$

The medium used in the cytotoxicity assays was RPMI 1640 containing 10% heat-inactivated foetal calf serum. This serum source was free of antibodies to IBR, HSV, or VSV viruses. Treatment of PMN to exclude cytophilic antibody. PMN from antibody free donors were preincubated in serum free medium (RPMI 1640) at 37 °C for 30 min as described by Goldstein et al.[16], pretreated at pH 4.0 at 0 °C for 1 min[18] or pretreated with 1% trypsin for 30 min at 37 °C[17] to remove putative cytophilic antibody. After treatment, the PMN were washed 3 times prior to use in the cytotoxicity assays.

Results and discussion. Bovine mammary neutrophils (PMN), were incubated with uninfected or virus infected cells both in the presence and absence of rabbit complement. The results, shown in table 1, indicate that bovine kidney cells, upon infection by IBR virus became susceptible to destruction by PMN and complement. Low levels of cytotoxicity in the absence of PMN was sometimes observed at the highest concentrations of complement used, but PMN alone exerted no appreciable cytotoxic activity (always less than 1% specific lysis). The bovine kidney cells showed increasing susceptibility to CDNC with time after infection (data not shown). Other leukocyte types were also tested for their ability to cooperate with C to lyse virus infected target cells. Peripheral blood lymphocytes, depleted of adherent cells, were ineffective but mammary gland macrophages gave low levels to CDNC that varied between 20 and 50% of that expressed by PMN.

Since IBR virus is a common viral pathogen of the bovine species, the possibility had to be considered that the cytotoxicity was attributable to cytophilic anti-IBR antibody adhering to the PMN, or present in the foetal calf serum (FCS), or complement source giving ADCC or complement facilitated ADCC[6, 19]. These possibilities were considered