different banding patterns were obtained from those reported here. SDS treatment was not employed and so separation was due partly to the molecular sieving effect of the acrylamide and partly to differences in charge/mass ratio of the sample proteins. Electrophoretic separation of SDS protein complexes on the other hand is due entirely to molecular sieving. Also, previous work employed gels of hich acrylamide concentration, and the large protein species observed in the present study may have failed to enter the gel matrix.

The use of SDS polyarylamide gradient gels has resulted in good resolution of protein species secreted in response to low doses of agonists and has enabled molecular weights to be determined. This in turn could greatly enhance the subsequent identification of protein species.

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Antigenic differences between a primary hamster lymphosarcoma and its liver metastases

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Summary. An antiserum was raised in rabbits against a primary metastasizing lymphosarcoma (ML) of the hamster. This was made tumor-specific by absorption with normal hamster tissue extracts. Immunoglobulin-G was prepared and tested for its cytotoxicity towards cells derived from the primary tumor and its liver metastases. The ML-specific IgG was found to be 2-5 times more cytotoxic for cells derived from the primary tumor compared to cells obtained from liver metastases.

Primary tumors are composed of a population of cells which are demonstrably heterogeneous as judged by several criteria of biological behavior, especially metastatic ability[3,4]. Although cells from both non-malignant and malignant tumors may be found in the vascular system, only a very small proportion of these circulating cells survive and successfully form secondary deposits, with the majority being destroyed by the hosts immune system. The metastatic cells have been shown to differ from cells of the primary tumor in susceptibility to drugs, adhesive interactions with different tissues and in their antigenicity[5-7]. Previous investigations carried out in this laboratory on 2 hamster lymphosarcomas have shown that the metastasizing (ML) form is less antigenic than the non-metastasizing (NML) form, and that this is attributable to a loss of antigens from the surface of the ML cells[8]. In this communication, we report our recent findings of antigenic differences between a primary lymphosarcoma in hamsters and its liver metastases.

Materials and methods. Tumors. The metastasizing lymphosarcoma (ML)[9] was maintained by serial transplantation in 2-4-month-old inbred Syrian Cream hamsters. Animals were injected s.c. with 0.2 g of finely chopped tumor tissue in 0.5 ml of medium 199. The biological characteristics of the tumor have been well investigated and its metastatic ability has been previously demonstrated in this laboratory[8,10].

For immunization of rabbits, unicellular suspensions of the tumor were prepared by collagenase disaggregation as described by Guy et al.[11]. Viable tumor cells were separated from non-viable tumor cells and erythrocytes by sedimentation at 1500× g for 15 min in 10 ml of Ficoll 400 (6.35% w/v, Pharmacia)-Hypaque (9.97% w/v, Winthrop Laboratories, Newcastle upon Tyne) as described by Mavligit et al.[12]. Tumor cell suspensions with viability in excess of 95% were used for immunization and in all subsequent procedures of binding and cytotoxicity testing in vitro. Tumor cell content of the preparations was determined as described previously[10] and was approximately 94% for both tumor types.

Target cells. Viable tumor cells obtained from collagenase-mediated disaggregation of primary ML tumors were used as target cells in the cytotoxicity tests. Secondary tumor cells were prepared from livers of the same animals using collagenase as described by Guy et al.[11]. Viable tumor cells were purified from erythrocytes and non-viable cells by flotation on Ficoll-Hypaque discontinuous gradient as described above.

Preparation and purification of antiserum. Half-lop male rabbits (~ 1.8 kg b.wt) were injected every 2 weeks with

ML tumor cells in equal volumes of Freunds complete adjuvant. Altogether 7 injections were given over a period of 3 months with increasing numbers of cells (10, 20, 25, 30, 50, 120, 360×10^6 cells), one half of each aliquot being given i.m. and the other s.c. The rabbits were bled 7-10 days after the final injection and sera were stored at −70 °C until further analysis.

Protein from ML tumors and from normal hamster liver, kidney and spleen was solubilized with 3 M KCl, the method employed was as described by Shah and Dickson[13]. The antiserum was characterized by immunodiffusion. The undiluted antiserum was placed in the central reservoir of Ouchterlony gels and 3 M KCl solubilized tissue protein at 10 mg/ml (PBS pH 7.2) in surrounding wells. The plates were left at 4 °C for 24 h. The antiserum was made tumor-specific by absorption against 3 M KCl extracts (freeze dried) of normal liver, kidney, spleen and normal hamster serum. Each absorption was carried out with 4 mg tissue protein/ml of antiserum for 1 h at room temperature and then for 24 h at 4 °C. The resulting antigen-antibody precipitate was removed by centrifugation at $105{,}000 \times g$ for 1 h.

Preparation of IgG. Immunoglobulins were prepared using the method described by Shah[14]. The 1.6 M $(NH_4)_2 SO_4$ precipitated immunoglobulins were loaded on a Sephadex G200 column (gel bed 33×2.6 cm, Pharmacia Fine Chemicals, Uppsala, Sweden) and proteins eluted at 4 °C with 10 mM Tris/HCl, pH 7.45, containing 150 mM NaCl at an elution rate of 8 ml/h (2 ml fractions). Fractions corresponding to IgG (Ve/Vt = 0.46-0.48), as confirmed by the elution of commercial rabbit IgG marker (Miles Seravac), were pooled and stored at −70 °C until use.

Cytotoxicity assays. The cytotoxicity of the purified anti-ML IgG preparations was determined as follows: 0.2×10^6 tumor cells were pelleted in a tube to which 0.1 ml of IgG in PBS and 0.1 ml of guinea-pig complement (Burroughs-Wellcome) was added, and the cells incubated for 60 min at 37 °C. At the end of the incubation, 40 µl of Trypan Blue (0.4% solution in PBS) was added and the tubes left at room temperature for 10 min. Cell counts were made in an improved Neubauer haemocytometer. Cytotoxicity is given as percentage of stained or dead cells in each tube. For each antiserum tested, duplicate tubes were set up and 4 counts were made for each tube. In each experiment, appropriate controls were maintained using normal rabbit IgG plus complement.

Results and discussion. The cytotoxicity of tumor-specific anti-ML IgG was tested in 3 separate experiments. In each experiment cells derived from the primary tumor (1°) and its liver metastases (2°) were used as target cells. The IgG preparations were cytotoxic to both 1° and 2° cells, but consistently the cytotoxic effect was much lower in the case of the 2° cells. In the table, the differences in the cytotoxicity are given in the form of a 'differential cytotoxicity index' (DCI), which represents the ratio between the difference in cytotoxicity of IgG-treated and control (incubated with normal rabbit IgG plus complement) 1° cells and that between IgG-treated and control 2° cells. Although the DCIs showed much variation between experiments, the results show that anti-ML IgG is 2-5 times more cytotoxic to cells of the 1° tumor than the 2°.

Differential binding of anti-ML IgG to primary and secondary cells of ML lymphosarcoma

Experiment No.	Primary tumor cells ΔC1° (% cytotoxicity anti-ML IgG-control)	Secondary tumor cells ΔC2° (% cytotoxicity anti-ML IgG-control)	Differential cytotoxicity index (DCI)
1	10.7	2.1	5.1
2	11.7	4.04	2.9
3	8.6	5.3	1.6

$DCI = \frac{\Delta C1°}{\Delta C2°}$ where C represents percent cytotoxicity, 1° primary tumor cells and 2° metastatic cells from liver of same animal. In each case, control cells were treated with normal rabbit IgG plus complement.

It may be suggested on the basis of these results that 1° and 2° cells share certain surface antigens, but these occur in much reduced amounts on the cells of the 2° tumors compared to the primary. Such reduced expression of surface antigens could be a mechanism by which a proportion of the disseminated cells are able to evade the immune surveillance of the host and form secondary growths. These results are compatible with the findings of Fogel et al.[15] that lymphocytes sensitized against cells from the 1° growth are significantly less cytotoxic to cells obtained from secondary deposits in lungs. Previously we demonstrated using the hamster lymphosarcoma system that the non-metastasizing lymphosarcoma did share surface antigens with the metastasizing lymphosarcoma, although in the latter only about 40% of the full complement of antigens could be detected[8]. In this context, Pimm and Baldwin[16] have demonstrated that primary methylcholanthrene-induced rat sarcomas can also be antigenically distinct from recurrent tumors arising at the same site after surgical removal of the primary tumor. These findings point to the multifocal origin of tumors and caution is necessary in designing active immunotherapy for the treatment of recurrences or metastatic deposits.

Note added in proof: Since we had previously shown that ML and NML tumors share certain surface antigens[8], we also tested anti-NML IgG on 1° and 2° cells. The cytotoxicity indices were higher than those obtained with anti-ML IgG. This is probably due to the higher titre of tumor-specific antibodies in the anti-NML serum. In this experiment also the anti-NML IgG was 3 times more cytotoxic to ML 1° than to 2° cells (DCI = 3.1), providing additional evidence for shared but reduced antigenic expression on metastatic cells as compared with cells of the primary tumor.

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