In vitro Immunoreactivity Against Ethynitrosourea-Induced Tumours of the Nervous System in the Rat

DRUCKREY et al.[1] demonstrated that tumours of the nervous system were selectively induced in the offspring of pregnant rats given a single i.v. injection of ethynitrosourea (ENU). Using this method of tumour induction we have studied the reactivity of lymphocytes and sera of tumour-bearing animals against their autologous tumour.

ENU, 50 mg/kg body weight, was injected into the tail vein of 12 pregnant, inbred, DA Agouti rats at the 15th day of gestation. When the offspring developed neurological signs, they were housed in individual cages, observed daily and killed in the preterminal stage. Tumours, blood and spleens were obtained for study. Diagnosis of the tumours was made by conventional histopathological methods. Tumour and spleen cell suspensions were prepared by gently teasing the tissue in tissue culture medium 199 enriched with 20% inactivated foetal calf serum and washing the cells twice; the lymphocytes were separated from the spleen cell suspension by centrifugation in a Hypaque-Ficoll gradient[2]. The viability of the tumour cell suspension, as assessed by trypan blue dye exclusion, varied from 20–90% (mean 50%) and the lymphocyte viability was more than 90%.

Autologous lymphocytes were cultured in Pulvertaft teflon ring chambers with tumour cells in a total cell ratio of 2.5:1 and observed daily by phase contrast microscopy for evidence of cytotoxicity over 4 days[3]. All tests and controls, with normal homologous spleen lymphocytes and medium 199 only, were performed in triplicate.

Tests for serum antibodies against tumour cell surface membrane were done by standard 'sandwich' immunofluorescence[3,4] using a fluorescein-isothiocyanate-labelled rabbit anti-rat globulin with a fluorescein: protein molar ratio of 4.0 and a globulin concentration of 1.9% absorbed with human liver powder, normal mouse serum and normal rat kidney.

26 out of 27 surviving rats developed tumours of the nervous system 121–342 (mean 202) days after birth; there were multiple tumours in 3 animals. 18 were gliomas of the central nervous system of which 7 were intracerebral and 11 intraspinal; these comprized 7 astrocytomas, 5 mixed gliomas, 1 oligodendroglioma, 1 ependymoma and 4 unclassified gliomas. There were 11 schwannomas of the peripheral nervous system, 7 of which involved the trigeminal nerve. Lymphocytic infiltration was present in 8 of the 18 gliomas but absent in all 11 schwannomas.

The lymphocytes of 16 out of 18 rats with gliomas were cytotoxic to autologous tumour cells in tissue culture; while the tumour cells grew cell in control cultures, in those cultured with autologous lymphocytes there were only a few cells attached to the coverslip and these often had a degenerate appearance. (Figure). In contrast none of the lymphocytes of rats bearing schwannomas showed any cytotoxic effect on autologous tumour cells. Also, the lymphocytes of the 3 rats which developed both a glioma and schwannoma were cytotoxic to autologous glioma cells but not to autologous schwannoma cells. All tests for serum reactivity against tumour cell surface membranes by immunofluorescence in 6 gliomas and 3 schwannomas were negative. In a limited cross-reactivity study by lymphocytotoxicity, the autologous-positive lymphocytes of a rat with glioma were inactive against 2 other autologous-positive gliomas and 1 schwannoma.

The data show that whereas in vitro anti-tumour lymphocytotoxicity is present in the majority of rats with gliomas, it is not demonstrable in rats with schwannomas. These findings are parallelled by histological evidence of lymphocytic infiltration, in some of the gliomas and its absence in the schwannomas. Also others have shown that while ENU-induced rat schwannomas transplanted subcutaneously in syngeneic rats grow well, the cor-

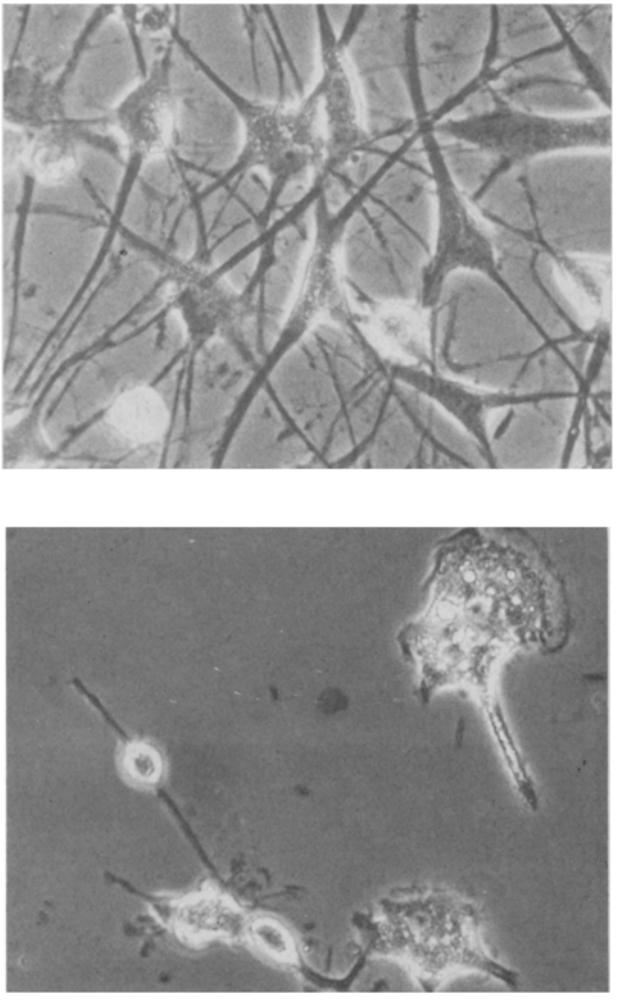

Fig. 1. Phase-contrast photomicrography. ×840. a) Cultured 5-day monolayer of astrocytoma cells from rat 9. b) Parallel culture destroyed by autologous spleen lymphocytes.

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responding gliomas are not readily transplantable[5-7]. These observations suggest that ENU-induced rat gliomas are strongly antigenic while the schwannomas are weakly or not antigenic[8,9].

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Résumé. L'éthylnitrosurée, injectée à des rates portantes, produit des tumeurs par voie placentaire dans le système nerveux de leurs descendants. In vitro, les lymphocytes spléniques des rats atteinst de gliomes furent cytotoxiques envers les cellules autologues de ces tumeurs, mais inactifs envers les cellules des schwannomes autologues. L'infiltration a eu lieu dans quelques gliomes, mais ne s'est pas produite dans le schwannomes.

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COGITATIONES

Are There any Nonspecific Large Molecule-Large Molecule Interactions?

In 1960[1] I was concerned with convincing my readers that some of the 'nonspecific' lectins extracted from plant seeds (invertebrate lectins had not yet been studied) were not so nonspecific after all. Writing to-day I should say, 'We have discovered the specificity of some plant lectins; here is some of the evidence'.

It is well known that the first lectin found to be blood group specific was the anti-A from Lima beans[2,3]. Elsewhere I have described the train of thought that led me to this discovery[4]. This lectin also precipitated specifically with blood group A substance. By studying the inhibition reactions of anti-A lectins, MORGAN and WATKINS[5] were able to conclude that the terminal unit of the polysaccharide chain determining the specificity of the A substance was N-acetyl-D-galactosamine. Since then a number of lectins, plant and invertebrate animal, have been studied, and SHARON and LIS[6] give a list of 18 such agglutinins that have been obtained in highly purified form. In the case of 15 of these the sugar specificity (presumably the terminal unit of the polysaccharide grouping with which they react) has also been determined. In addition to the L-galactose listed by SHARON and LIS as reacting with the lectin of *Ricinus communis*, BOYD and WASZCZENKO-ZACHARCZENKO[7] found 3 other sugars of Mäkelä's group 2 to inhibit as well or better. Sugars of group 3, however, which inhibited the 'nonspecific' lectin of *Bauhinia purpurea* var. *alba*, did not inhibit the *Ricinus* lectin at all. Therefore, although the complete structures of the receptors for these two lectins have not been elucidated, it is clear that each has a specificity, and that the two specificities are different.

Lectins probably came to be classified as 'nonspecific' because ealier work in immunology, always preeminently an applied science, had led to a narrow definition of specificity. Antibodies were considered useful if they reacted exclusively, or nearly so, with just 1 antigen. When it was attempted to produce immune agglutinins for the blood group antigens such as A, B, M, and N, the first step after injecting and bleeding the animals was to absorb out of the serum the 'nonspecific' agglutinins that reacted with all human bloods, leaving the agglutinins specific for A B, etc. The unwanted 'unspecific' agglutinins, more numerous and more abundant, which doubtless possess their own specificities, were labeled 'non-specific', absorbed out, and thrown away.

The haemagglutinating action of *Ricinus* extract was described before erythrocyte agglutinins were demonstrated in animal blood[4], soon after specific bacterial agglutination had been discovered. But *Ricinus* extracts agglutinate the erythrocytes of human beings of all blood groups, and of many species of animals[4], so this agglutinin was labeled 'nonspecific', and until recently little work was done on it, although LANDSTEINER had found it to agglutinate pigeon erythrocytes much better than horse erythrocytes. LANDSTEINER was well acquainted with the earlier work with plant agglutinins, and carried the work forward himself. He wrote a paper on 'Pflanzliche Hämagglutinine' which reached the stage of page proof, but was never published[8].

The haemagglutinating action of lectins is not the only interesting thing about them. Certain lectins are mitogenic[6], and this property has played a part in the important studies on the relationship between chromosome abnormalities and human diseases. Lectins have been found that are specific for tumor cells[6], greatly increasing interest in these substances. See also the recent Conference at the N.Y. Academy of Sciences[9].

Why do I now say it should have been obvious that all lectins have a specificity? Basically, because I think that lectin-antigen reactions, like antibody-antigens, involve surface configurations of limited size, as in fact do nearly all large molecule-large molecule interactions[10]. Actually it is difficult to imagine how 2 large molecules of the same overall charge could interact in any other way. Since these

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