Table 1. Cloning efficiency (CE) of 8701-BC cells in 0.6% agar after selection by adhesion onto type V collagen substrates

Cell subpopulation	Cell viability (%)	No. of assays	Mean CE
	100	16	0.044*
V+	100	20	0.030
V−	27	20	0.112

*CE of the parental 8701-BC line [4].

at a concentration of 2000 viable cells/dish and incubated at 37°C in a 5% CO_2 atmosphere for the appropriate time, after which CE values were calculated by the formula:

$$CE = \text{colony number/initial cell concentration} \times 100,$$

as described elsewhere [4].

Table 1 reports the viability and CE values of 8701-BC cells selected in the presence of type V collagen, compared with previous results obtained with the parental cell line [4]. Approximately 73% of V− cells clearly undergo cell death after 24-h incubation, as revealed by the lowering of Trypan blue exclusion after staining. Interestingly, the surviving fraction of the V− cell subpopulation, once allowed to grow in 0.6% agar, shows a more than 3-fold increase of clonal proliferation versus V+ cells. It is noteworthy also that the CE of the unselected 8701-BC line is lower, although to a minor extent, than that of V− cells. Consequently, the data obtained indicate that type V collagen exerts a clonal selection on the heterogeneous 8701-BC cell line, allowing the adhesion and survival of the potentially less malignant cells (by involvement of the 67 kDa [10] and/or other surface receptors), and inhibiting the propagation of cells endowed with a stronger neoplastic aggressiveness.

It is well known that in development and cancer, the extracellular matrix (ECM) undergoes massive compositional changes, both quantitative and qualitative which, in turn, influence the state of cell differentiation by modifying the multisignal network of interactions. We have reported previously that individual collagen species are able to elicit diverse, and in some cases opposite, responses by DIC cells *in vitro*. In particular, type V collagen was found to be an anti-adhesive (in part), antiproliferative and anti-locomotory substrate, even when used as hybrid matrices with type I collagen [1,7,8]. A similar inhibitory effect by this collagen type was also proven in other cell systems, both normal and transformed ([11] and references therein). The data here reported show another interesting "anti-cancer" property of collagen type V, at least for the cell line under study.

Moreover, the present results give further support to the hypothesis that the over-deposition of type V collagen occurring in the stroma of DIC [12,13] may be regarded as a true defensive host reaction. This could negatively regulate the progression of DIC *in vivo*, being one of the "instructive" ECM signals which concomitantly contributes to directing the tumour cell population towards different levels of malignancy by modulating gene expression and phenotypical selection.

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Acknowledgements—This work was supported by grants from AIRC and MURST (R.S. 60% and 40%) to Prof. Ida Pucci-Minafra. We wish to thank COBS for the hospitality in its laboratory.

European Journal of Cancer Vol. 30A, No. 9, pp. 1401–1403, 1994.

0959-8049/94 $7.00 + 0.00

0959-8049(94)00235-5

Intracranial Germ Cell Tumours Presenting With Hypopituitarism. Successful Treatment with Chemotherapy Alone

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INTRACRANIAL GERM cell tumours (GCT) represent only 0.3–3.4% of all primary intracranial tumours in children, and more than 95% of them present with the consequences of a mass lesion in either suprasellar or pineal regions. Pineal tumours

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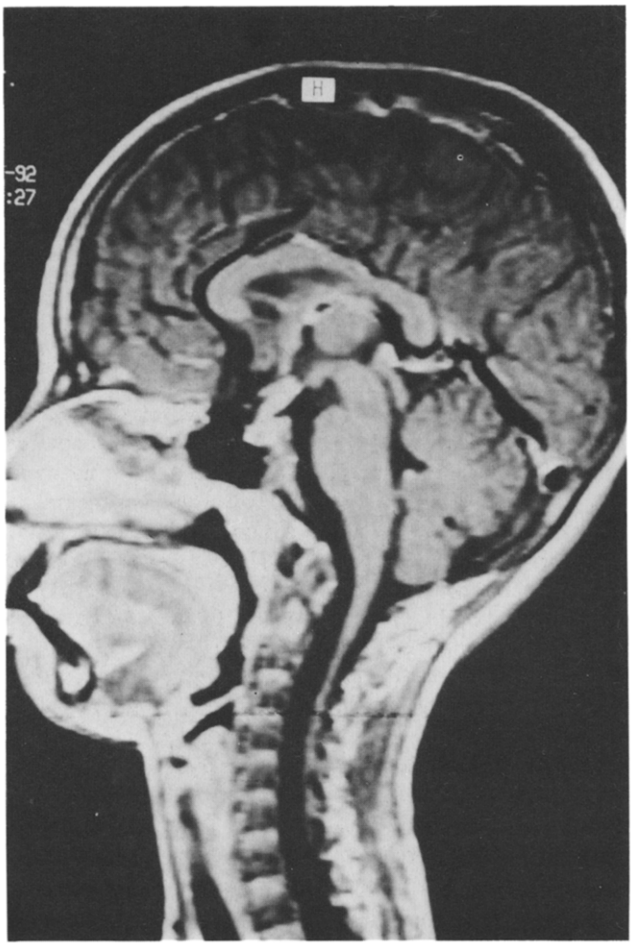
Revised 11 May 1994; accepted 7 June 1994.

(a)



(b)

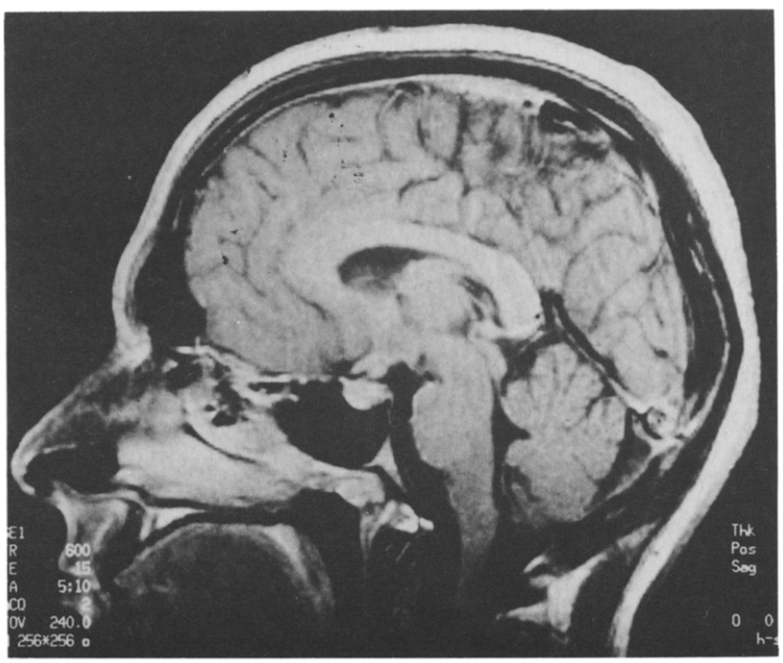


Figure 1. (a) MR brain scan of case 1 pretreatment demonstrating mass at tuber cinerium. (b) MR brain scan of case 2 showing thickened pituitary stalk.

usually present with hydrocephalus and precocious puberty, and suprasellar tumours with endocrine failure or visual symptoms. Usually a mass is readily detected with computed tomography (CT) or magnetic resonance (MR) scanning and the diagnosis made by biopsy, but sometimes imaging changes are subtle and biopsy hazardous. Here we present 2 cases of intracranial GCT presenting with insidious hypopituitarism, and diagnosed by a combination of MR scanning and raised levels of cerebrospinal fluid (CSF) human chorionic gonadotrophin (HCG).

The first, a girl aged 7 years presented with growth failure. Growth hormone (GH) deficiency was diagnosed 5 years later at 12 years. GH was not available in India, where the patient lived at that time, so no further treatment was undertaken. The family moved to London where the patient complained of polyuria and polydipsia, and was noted to be below the third centile for both height and weight. Endocrine function tests revealed GH deficiency, hypothyroidism and diabetes insipidus, and she was treated with genotropin, thyroxine and dd-arginine vasopressin (ddAVP). There was a dramatic growth response and her symptoms resolved. MR imaging revealed a thickened pituitary stalk tumour [Figure 1a] but biopsy was considered to pose an unjustifiable risk to pituitary integrity. Serum and CSF alfa-fetoprotein (AFP) levels were within the normal range, but serum and CSF HCG levels were 4 and 11 U (normal range <2 U), respectively. A diagnosis of GCT was made. She was treated with JEB chemotherapy [carboplatin (area under curve, AUC 6) day 1, etoposide 120 mg/m^2 intravenous (i.v.) days 1 to 3 and bleomycin 30 mg i.v. day 1 carboplatin dosage is calculated by a nomogram related to renal excretion as the area under the plasma concentration against time curve, AUC [1]], repeated for four cycles 3-weekly with appropriate supportive measures. Treatment was well tolerated with no hospital admissions. Both serum and CSF HCG levels and MR scan returned to normal after 4 cycles. No radiotherapy was given. She remains well, on endocrine replacement, 15 months after the end of treatment.

The second patient, male, presented at age 13.5 years, with polyuria and polydipsia. Diabetes insipidus was diagnosed and ddAVP commenced. Plasma thyroid stimulating hormone (TSH), follicle-stimulating hormone (FSH) and uteinising hormone (LH) levels were normal, but GH response to hypoglycaemia was poor. A cranial MR scan showed a small mass (Figure 1b) at the tuber cinereum. Biopsy was considered unjustifiably risky and treatment was deferred. After 6 months, growth was slowing and he was started on GH replacement. Six months later, hydrocortisone and thyroxine were started after low basal-free thyroxine and cortisol levels. Serum and CSF HCG levels were 13 and 105 U, respectively, and a diagnosis of GCT was made. Serum and CSF AFP levels were normal. As in case 1, treatment was with four courses of JEB chemotherapy only. It was well tolerated, and tumour markers and MR scan returned to normal. He remains well on hormone replacement 21 months after completing treatment. As yet, neither anterior nor posterior pituitary function has recovered and he has recently started testosterone replacement.

Most intracranial GCT are diagnosed histologically following imaging and biopsy. Our cases are unusual in that they presented at sites where the risks of biopsy were unjustifiable. The differential diagnosis of a mass in the pituitary stalk and tuber cinereum region includes craniopharyngioma, Langerhans cell histiocytosis, granulomatous disease (in particular, tuberculous granulomata) and GCT. Our cases demonstrate the value of CSF tumour markers when biopsy is not possible. The use of MR scanning is also important particularly in indolent disease: 5% of patients with germinomas have a 5-year delay in diagnosis [2]. Thus, the patient with idiopathic hypopituitarism merits MR scanning, visual field estimation and both serum and CSF AFP and HCG measurement [3], perhaps on more than one occasion.

Until recently, surgery followed by radiotherapy was considered "standard treatment" for intracranial GCT [4]. Using this approach, the prognosis in seminomatous GCT was fair, with a 5-year survival of approximately 50%, but patients with non-seminomatous GCT usually died of progressive tumour. Reduction in usage and dosage of radiotherapy is a well-recognised current objective in paediatric oncology. Amongst the benefits of reduced radiation for children's brain tumours are (a) less intellectual and developmental damage, (b) less pituitary/hypothalamic injury, with consequent need for hormone replacement therapy and (c) reduction in radiation-induced "secondary malignancies" [5]. The successful use of combination chemotherapy in the management of advanced extracranial GCT [6] means that it is now often used as first-line treatment for patients with intracranial GCT, with small series indicating a cure rate of at least 50% with chemotherapy alone [3]. We chose to use JEB chemotherapy in these two children because of the proven efficacy of this regime in paediatric GCT [1], and to avoid ototoxicity and nephrotoxicity.

In summary, pituitary stalk GCT may present with insidious hypopituitarism and without hydrocephalus or a "mass effect". New imaging techniques and CSF tumour marker levels have made these tumours easier to diagnose, when biopsy is considered hazardous. Poor results with conventional treatment (surgery and radiotherapy) and concern about the late effects of radiotherapy mean that chemotherapy is increasingly used as a first-line treatment. Patients with small tumours, like these, may be cured by chemotherapy alone. If there is local recurrence, there is a substantial chance of cure with further chemotherapy, then radiotherapy.

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Acknowledgements—We thank Dr M Liberman for allowing us to publicise details of patient 1 and Professor Alan Horwich for his very helpful comments.