Short Communication

EFFECT OF *PASTEURELLA PNEUMOTROPICA* ON THE GROWTH OF TRANSPLANTED WALKER SARCOMA CELLS

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THE Walker sarcoma has been used at the Institute of Cancer Research (ICR) for many years to study the effect of potential anti-tumour agents. The tumour was routinely maintained in Wistar rats by transplanting cells derived either from tissue culture or growing tumours. One group of transplanted tumours derived from the same batch of tissue culture behaved abnormally, by regressing spontaneously. These cell cultures were examined bacteriologically and a pure growth of *Pasteurella pneumotropica* of the indole-negative group (Simmons & Simpson, 1977) was isolated. After treatment with streptomycin, the cells, when transplanted into rats, grew normally, whereas untreated cells from the same batch either failed to grow tumours or the tumours which did grow regressed spontaneously.

This effect was studied further using deliberate mixtures of Walker tumour cells and *Pasteurella*. The presence of the bacterium can be instrumental in inhibiting tumour growth, but the immunological response of the host animals is a prerequisite.

The animals used in this study were inbred Wistar/P.Cbi rats, both intact and T-cell deprived, inbred CBA/Ca mice, both intact and T-cell deprived, and random/*nunu* (athymic) mice. All animals were obtained from the barrier-maintained breeding colonies of the ICR. They were housed in polypropylene cages with stain-less steel tops and fed a standard pasteurized laboratory animal diet and water *ad libitum*.

The Wistar rats were T-cell deprived by the method of Davies, quoted by Harding *et al.* (1971) and the CBA/Ca mice by the method of Miller *et al.* (1963).

Tumour cells were derived from transplanted solid tumours. Such tumours were removed aseptically from rats and homogenized in medium 199 (Wellcome Reagents, London). Large clumps of cells were allowed to settle out. The supernatant was used to prepare the inocula for the experiments. No attempt was made to distinguish between dead and viable cells.

Either 4×10^5 or $1 \cdot 9 \times 10^6$ solid-tumour cells alone or in admixutre with *P. pneumotropica* (total vol. 0·5 ml) were injected s.c. into the inguinal region of Wistar rats. Mice were treated similarly but with only 1×10^5 cells in 0·2 ml of medium.

All animals were killed 14 days after inoculation. Previous experience with the Walker tumour has shown that animals without tumours at that time will not get them later and that animals with tumours are within a few days of death.

The strains of *P. pneumotropica* used were isolated from the Wistar rat colony, in which it is endemic. It had the same biochemical characteristics as the strain originally isolated from the cell cultures.

Fresh isolates were used in most experi-

ments because repeated subculture on blood agar can reduce the efficacy of Pasteurella in inhibiting tumour growth. Suspensions of P. pneumotropica were made in Medium 199 and adjusted to Brown's opacity tube 3, to give $\sim 3 \cdot 6 \times 10^9$ organisms per ml. This suspension was used as one fifth of the diluent in admixture experiments. For convenience only the Pasteurella was incubated with the tumour cells for up to 1 h at room temperature before injection into the test animals. When dead organisms were used the Pasteurella was killed by heating at 60°C for 1 h before its incubation with the tumour cells.

The results of the transplantation experiments in rats using cells derived from solid tumours mixed with P. pneumotropica are shown in Table I.

TABLE I.—Effect of adding P. pneumotropica to cells from solid Walker tumours transplanted into rats

| Rat type | Treatment | | No. of rats with tumours at 14 days/ No. tested |
	Cells	Pasteurella	
Intact	4×10^5	—	24/25
Intact	4×10^5	+	0/24
Intact	$1 \cdot 9 \times 10^6$	—	7/8
Intact	$1 \cdot 9 \times 10^6$	+	6/12
Deprived	4×10^5	—	6/6
Deprived	4×10^5	+	6/6

None of the intact rats given 4×10^5 tumour cells with live P. pneumotropica grew tumours in contrast to similarly treated T-cell-deprived rats. The growth of Pasteurella-treated tumours in deprived rats was however slower than that of tumours grown in control deprived rats injected with 4×10^5 tumour cells only; the tumours in the control deprived rats had a diameter of 2–3 cm 14 days after inoculation of cells twice as large as tumours from Pasteurella-treated rats. When $1 \cdot 9 \times 10^6$ tumour cells mixed with Pasteurella were injected, 50% of the intact rats injected grew tumours.

Tumour growth was unaffected in 6 rats given solid tumour cells mixed with dead

P. pneumotropica. When a strain of Pasteurella which had been subcultured several times was used with solid-tumour cells, the tumours grew, albeit slowly, in all 6 rats inoculated.

Tumours did not grow in intact CBA/Ca mice, but did grow in deprived CBA/Ca mice and random/nunu mice given either tumour cells alone or mixed with Pasteurella (Table II).

TABLE II.—Effect of adding P. pneumotropica to $1 \cdot 6 \times 10^5$ solid Walker-tumour cells injected s.c. into mice

Mouse type	Pasteurella	No. of mice with tumours at 14 days/ no. tested
CBA/Ca intact	—	0/4
CBA/Ca intact	+	0/4
CBA/Ca deprived	—	4/4
CBA/Ca deprived	+	4/4
Random/nunu (athymic)	—	2/2
Random/nunu (athymic)	+	2/2

The interaction between bacteria and tumours is one which has attracted much attention from the time of the early studies of Coley (1914) involving organisms as diverse as Listeria monocytogenes (Youdim, 1977), streptococci (Tokuzen et al., 1978), Micrococcus lysodeikticus (Verloes et al., 1979), Salmonella enteritidis (Ashman & Kotlarski, 1978) among many others. The experimental designs have varied from those comparable with the present studies in which bacteria and tumour have been mixed prior to transplantation, to others in which bacteria have been injected directly into a growing tumour or injected into a tumour-bearing animal at a site distal to the lesion (Zbar et al., 1978; Cohen et al., 1975). Variously successful attempts have been made to purify the part of the bacterium responsible. The extraction of such entities as lipid A (Kasai et al., 1961) a carbohydrate complex from Salmonella enteritidis (Shapiro, 1940) and the demonstrations of their anti-tumour potentiality stand out.

There can be little doubt that some of the tumoricidal effects, particularly those

in which contact between tumour cells and the bacterium or its product was achieved, were due at least partly to direct toxicity, a mechanism which cannot be excluded in the present study. Given that any of T lymphocytes, non-T-lymphocyte killer cells, macrophages and eosinophils might have the potentiality to be locally cytotoxic in high concentrations, the mechanistic possibilities after introduction of a bacterium into this mélange of cells are numerous. There could be (1) non-specific enhancement of a specific anti-tumour response, (2) augmentation of specific antitumour immunity due to cross reaction between bacterial and tumour antigens, (3) generation of a physiological environment which is inimical to tumour growth as a result of the specific anti-bacterial response, or (4) the same as 3 but due to a non-specific (non-immunological) reaction to the bacterium.

The disentanglement of these possibilities in such a manner as to lead to rational attempts at tumour immunotherapy, so-called, has not proved easy, and it could be argued that the whole field is falling into some disrepute in consequence. In the present studies the effect observed is broadly dependent on the host animal having an intact immune system, as previously described by Tokunaga *et al.* (1978) for the effect of BCG.

Whether the immunity involved in the present study is anti-tumour or antibacterial or both or neither has not been resolved. The bacterium involved is a commensal organism in the rats used and it could be that some kind of Shwartzman reaction (Shwartzman, 1928) occurs at the site of introduction of the tumours, as has been suggested by Shapiro (1940) in relation to the effect of an extract of *S. enteritidis* on rat tumours. If this were true the anti-*Pasteurella pneumotropica* immunity of the tumour-bearing animals would be an important component. It is noteworthy in this context that some retardation of tumour growth did occur in deprived rats implanted with tumour mixed with *Pasteurella*, an effect which

might be anticipated in that pre-existing immunity (in the present instance anti-*Pasteurella*) may be retained to some extent despite the deprivation process (Davies *et al.*, 1964).

There is the possibility of an immune response against the Walker tumour, but it has been shown that the tumour grows at a similar rate in normal and T-cell-deprived rats (Connors & Davies, unpublished) and it is thus not obvious that there is a specific cell-mediated host antitumour response to be augmented.

These studies, though incomplete, show clearly that heavy contamination of transplanted tumours by commensal bacteria can lead to graft failure. Whether such an effect has any significance for the reduction of existing tumours remains to be seen.

Attempts to influence the outcome of chemotherapy of melanomas by the use of vaccinia virus injected directly into tumours carried by previously vaccinated individuals (Roenigk *et al.*, 1974) is perhaps germane to this argument. Such attempts have not been generally successful, but better description of the immune status of the host, and better prediction of its influence on the reactions which might follow introduction of previously recognized antigen, might help in obtaining better results.

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