Eur J Cancer Clin Oncol, Vol. 24, No. 6, pp. 1075–1078, 1988.
Printed in Great Britain

0277–5379/88$3.00+0.00

Letter to the Editor

Possible Therapeutic Use of Modified Anaerobic Bacteria in Cancer

LEON M. COBB

Division of Experimental Pathology and Therapeutics, MRC Radiobiology Unit, Chilton, Didcot, Oxon OX11 0RD, U.K.

AN EXPANDING tumour cell population in an organ sooner or later reaches a point at which the host blood supply is unable to support further growth without some tumour cells becoming temporarily, or permanently, hypoxic. Further expansion of the cell population is usually accompanied by the development of foci of necrosis as the tumour fails to find adequate vascular support from the host. The oxygen tension in these necrotic areas can be expected to be very low or zero [1]. It is precisely these conditions of minimal to zero oxygen together with tissue necrosis in which many anaerobic bacteria can thrive.

Möse and Möse, and others [2–5] have screened a number of sporing clostridia for their ability to proliferate in experimental tumours. One such anaerobe, *Clostridium butyricum* strain M-55, isolated by Möse and Möse from a soil sample, was shown by them, and subsequently by others, to produce extensive lysis of transplanted and chemically induced tumours in a variety of laboratory animals [4, 6, 7]. In a typical experiment Engelbart and Gericke [6] implanted melanoma subcutaneously in hamsters and when the tumour was 1 cm in diameter (18–22 days) 10^8 *Cl. butyricum* spores were injected intravenously (i.v.). The spores became trapped, and subsequently germinated, in the tumours of 10 out of 10 animals, and within 3 days the tumours were liquified. Subsequently the skin over the tumours ulcerated discharging the fluid contents, which contains vegetative forms of *Cl. butyricum*. At that point some of the tumour-bearing animals died, probably from the combined effects of the bacterial toxins and the products of tumour necrosis [6]. In other experiments the periphery of the tumour was not completely lysed and this led to the regrowth of the tumour. Some workers have observed that a tumour which regrows after oncolysis may again undergo lysis due, it is thought, to the persistence of spores after the initial lytic episode [8]. In a small clinical trial von Heppner and Möse injected 10^{10} strain M-55 *Cl. butyricum* spores into the carotid artery of 20 patients with brain tumours [9]. Because there was only a small number of patients, and amongst these the tumour type differed, it was not possible to assess clearly the benefit of this form of treatment, but this trial did show that in most of the patients the spores settled in the tumours, germinated and produced tumour lysis.

Over the years attempts to supplement the therapeutic action of anaerobes have included local hyperthermia [10] and regimens of radiation and drugs directed towards the cells at the periphery of the tumour [8, 11, 12]. These combinations have occasionally produced cures in experimental tumours [13]. It is perhaps not surprising that this form of treatment remains a minority interest. The trapping and subsequent proliferation in tumours of clostridial spores injected into the blood stream is a random process, and we have little knowledge of the factors which influence the retention of anaerobes within tumours. Even when germination has occurred the subsequent tumour lysis is uncontrolled, relying as it does upon cytotoxin production by the organism and possibly also competition for available nutrients between the bacteria and the tumour cells [8].

Accepted 11 February 1988.

We have been looking again at the use of anaerobes in cancer therapy and conclude that advances in recombinant DNA technology made in the last few years may perhaps provide a means for refining the use of anaerobes in tumour therapy and diagnosis. For example, an innocuous anaerobe proliferating in a tumour and carrying plasmids producing high levels of an enzyme capable of activating an otherwise inactive cytotoxic drug (prodrug) might produce a valuable degree of tumour necrosis. Control of local concentration of active drug would be derived from the rate of administration of the prodrug. With the general approach of genetic engineering in mind the tumour localizing ability of the non-sporing anaerobe *Bacteroides nodosus* has been examined. *B. nodosus* has the advantage that it is only pathogenic in special circumstances, and then only in the sheep ('foot rot') and ox (dermatitis). Additionally, because it does not spore and is sensitive to the common antibiotics, it can quickly and easily be eliminated from the host should the need arise. *B. nodosus*, strain 405, was kindly supplied to us by Dr. C.M. Thorley of the Wellcome Research Laboratories, Beckenham, Kent.

In a series of studies the persistence of *B. nodosus* 4 days after i.v. injection was examined in tumour-bearing mice. In addition, the possibility was explored that the simultaneous injection of a non-pathogenic coccus (facultative anaerobe) could improve the likelihood of the *B. nodosus* becoming established. Two transplantable mouse tumours, an osteosarcoma (A44) and a hepatocarcinoma (A112), syngeneic to the male CBA/H mice used in the study, were prepared by an injection of 10^6 viable cells subcutaneously into both flanks. When the tumours were 0.5–0.8 cm in diameter, which was 10–15 days after the injection of the tumour cells, the animals (44 mice), were injected with the test bacteria.

B. nodosus was cultured anaerobically on a medium consisting of 1% w/v proteose peptone, 1.5% w/v tryptone, 1% w/v liver digest, 0.5% w/v NaCl, 3.5% w/v agar [14]. The bacteria were harvested in the active growth phase and 10^8 injected i.v. in 0.1 ml nutrient broth. The non-pathogenic coccus was cultured on nutrient agar and similarly injected (Table 1). The coccus was included with some injections because pilot studies had indicated that its presence in a tumour might encourage the establishment of *B. nodosus*, possibly by reducing the local pO_2. For each of the two tumour types 22 mice were injected with bacteria and examined as follows. Culture for intratumour *B. nodosus* was carried out on six mice injected with *B. nodosus* alone and on six mice injected with *B. nodosus* plus coccus. Examination of normal organs (spleen, liver and kidney), but not tumour, for *B. nodosus* and coccus was carried out on 10 mice injected with *B. nodosus* plus coccus. The examination of tumour and normal organs for bacteria was made 4 days after bacterial injection. The tissues to be examined were gently fragmented and samples at two concentrations of supernatant were cultured anaerobically and the colonies counted and identified.

The tumour results are given in Table 1. All samples from the spleen, liver and kidney from the 20 mice were negative for both injected bacteria. The simultaneous injection of coccus had not improved the retention of *B. nodosus* by the osteosarcoma but may have been of assistance in the hepatocarcinoma. The results confirm the findings of previous workers in this field, that anaerobes will persist in tumours, presumably because of the necrotic, anoxic, conditions that exist. Because of the random nature of the trapping of circulating bacteria in the tumour it would seem likely that repeated injections might increase the percentage of tumour with positive growth. It could also be assumed that the larger the tumour mass the greater the likelihood of bacterial trapping.

By far the majority of cloned genes are available in plasmids compatible with the aerobe *Escherichia coli*. Nevertheless, plasmid transfer to *Bacteroides* has been achieved [15]. This opens up the possibility of putting into *B. nodosus* the DNA required to express a wide variety of antigens, enzymes, toxins and receptors. The administration of anaerobes carrying genetic information which is then expressed in malignant tissue may find application in the following ways:

(i) The bacteria could be engineered to export substances which are cytotoxic to certain tumour cells; for example, l-asparaginase. However, as with *Cl. butyricum* the amount of cytotoxin produced in this way would not be controlled.

(ii) Enzymes could be produced locally by the anaerobes (within the tumour) which would activate otherwise inactive injected drugs. For example, a high level of β-glucuronidase would produce locally the highly cytotoxic *para*-hydroxy analine mustard from the minimally toxic glucuronyl analine mustard. The latter compound is formed in the liver and subsequently circulates following the administration of the innocuous precursor analine mustard [16, 17].

(iii) Novel surface or secreted bacterial antigens could be used to form high affinity antibodies. The use of such antibodies would be of particular advantage in radioimmunodiagnosis as the anaerobe-produced antigen could be tumour specific. Additionally, one antibody would serve all tumour types. The possibility that *Cl. butyricum* (M-55) would have suitable natural antigens was explored

Table 1. *Positive culture of* B. nodosus *in tumours*

	Bacteria injected	
	B. nodosus	*B. nodosus* + cocci
Osteosarcoma (A44)	5/12* (3/6)	4/12 (3/6)
Hepatocarcinoma (A112)	2/12 (2/6)	9/12 (6/6)

*The tumour was transplanted bilaterally in each mouse. The results are expressed as the total number of tumours positive for *B. nodosus* and, in brackets, the number of mice with either one or two *B. nodosus*-positive tumours.

by Möse and Schwager [18] with the same purpose in mind.

(iv) Receptors, not necessarily antigenic, could be formed and accumulate locally. These would receive the appropriate injected ligand. This ligand could carry drugs, toxins or radionuclides. For example, locally formed bacterial avidin would retain injected ^{131}I-labelled biotin for the purposes of tumour imaging.

(v) Locally formed subsances could augment present day treatment of the patient. Human tumour necrosis factors (TNF) α and β have already been cloned by recombinant DNA methods [19].

In conclusion, we know that in many patients tumour is unique among tissues in providing an environment for the proliferation of anaerobes; and DNA technology allows us to programme such anaerobes to export a variety of substances. If we can now develop techniques for reliably trapping innocuous injected anaerobes in tumours we may just have another handle on the diagnosis and therapy of this disease.

Acknowledgements—Thanks are due to Dr. C.M. Thorley for much useful advice and Miss S.A. Butler for careful technical assistance.

REFERENCES

1. Vaupel P. Oxygen supply to malignant tumours. In: Peterson H-I, ed. *Tumour Blood Circulation*. CRC Press, 1979, 143–168.
2. Möse JR, Möse G. Onkolyseversuche mit apathogenen, anaeroben Sporenbildern am Ehrlich-Tumor der Maus. *Z Krebsforsch* 1959, **63**, 63–74.
3. Möse JR. Zur Beeinflussbarkeit verschiedener Tier-Tumoren durch einen apathogenen Clostridienstamm. *Z Krebsforsch* 1960, **63**, 447–455.
4. Möse JR, Möse G. Oncolysis by clostridia. I. Activity of *Clostridium butyricum* (M-55) and other non-pathogenic clostridia against the Ehrlich carcinoma. *Cancer Res* 1964, **24**, 212–216.
5. Fabricius E-M, Benedix A, Schneeweiss U, Schmidt W, Streller B. Bestimmung der onkolysierenden Wirkung von Klostridien am Hamster-A Mel 3-Modell. *Arch Geschwulstforsch* 1978, **48**, 538–551.
6. Engelbart K, Gericke D. Oncolysis by clostridia. V. Transplanted tumours of the hamster. *Cancer Res* 1964, **24**, 239–243.
7. Mehnert WH, Winkler J, Graffi A. Das anskeimungsvermögen von *Clostridium butyricum* Jena H8 in transplantationstumoren. *Arch Geschwulstforschung* 1979, **49**, 97–105.
8. Theile EH, Arison RN, Boxer GE. Oncolysis by clostridia. III. Effects of clostridia and chemotherapeutic agents on rodent tumours. *Cancer Res* 1964, **24**, 222–233.
9. von Heppner F, Möse JR. Onkolyse maligner Gliome durch apathogene Clostridien (stamm-M55). *Zbl Neurochir* 1966, **27**, 183–192.
10. Gericke D, Dietzel F, König W. Mikrowellenhyperthermie und Onkolyse durch Clostridien. *Naturwissenschaften* 1975, **62**, 541–542.
11. Dietzel D, Gericke D, König W. Tumourhyperthermie mit Hochfrequenz zur Verstärkung der Onkolyse durch *Clostridium butyricum* (Stamm M55). *Strahlentherapie* 1976, **152**, 537–541.
12. Dietzel F, Gericke D, Schumacher L, Linhart G. Combination of radiotherapy, microwave-hyperthermia and clostridial oncolysis on experimental mouse tumours. In: Streffen C, ed. *Cancer Therapy by Hyperthermia and Radiation*. Essen, Urban and Schwarzenberg, 1978, 233–235.
13. Geicke D, Dietzel F, König W, Rüster I, Schumacher L. Weitere Fortschritte bei der Onkolyse durch apathogene Clostridia. *Zbl Bakt Hyg* 1979, **243**, 102–112.
14. Thorley CM. A simplified method for the isolation of *Bacteroides nodosus* from ovine footrot and studies on its colony morphology and serology. *J appl Bact* 1976, **40**, 301–309.
15. Guiney DG, Hasegawa P, Davis CE. Plasmid transfer from *Escherichia coli* to *Bacteroides fragilis*: differential expression of antibiotic resistance phenotypes. *Proc Natl Acad Sci* 1984, **81**, 7203–7206.
16. Whisson ME, Connors TA. Cure of mouse bearing advanced plasma cell tumours with analine mustard. *Nature* 1965, **206**, 689–691.

17. Connors TA, Whisson ME. Cure of mice bearing advanced plasma cell tumours with analine mustard: the relationship between glucuronidase activity and tumour sensitivity. *Nature* 1966, **210**, 866–867.
18. Möse JR, Schwager J. Die Verwendung von Clostridien-Antigenen für eine serologische und autoradiographische Frühdiagnose von Tumoren. *Zbl Bakt Hyg* 1978, **242**, 512–516.
19. Pennica D, Nedwin GE, Hayflick JS *et al.* Human tumour necrosis factor: precursor structure, expression and homology to lymphotoxin. *Nature* 1984, **312**, 724–729.