HUMAN TUMOUR XENOGRAFTING AND RADIOIMMUNOTARGETTING

D. K. HAZRA, V. L. LAHIRI, I. P. ELHENCE, SHABAD SARAN and M. C. AGARWAL
Department of Medicine, S.N. Medical College, Agra 282 003, India.

ABSTRACT

The transplantability of human tumours especially breast carcinoma as xenografts has been examined in Swiss albino mice that had been immune-suppressed. Tumour takes have been noticed as early as on the eleventh day. Tumour targetting which has assumed great importance both for radioimmunodetection as well as radioimmunotherapy has been achieved in these models using ^{125}I labelled anti-HMFG-2 monoclonal antibodies. Such an immune-suppressed model is feasible and economical as compared to athymic nude mice.

INTRODUCTION

We are currently interested in a programme of research on economical techniques for the growth of human tumours in immune-suppressed mice and of creating *in vivo* model as well of infective lesion e.g. tuberculoma[1] to evaluate radiolabelled monoclonal antibodies or engineered cocktail[2] for radioimmunodetection (RID) with the hope that the work on tumours can be later extended to radioimmunotherapy (RIT).

The idea of human tumour implantation into experimental animal hosts is not new. Such xenografting is, in some sense, an extension of the many ways in which transplantation of animal tissues and organs to the human species was attempted as replacement therapy. Heterografting of human tissues, chiefly pioneered by Handler[3] has attracted growing interest and brought an increasing number of human tumours within the scope of *in vivo* experimentation. Such xenografts can be used for diverse investigations such as chemotherapy testing and study of tumour-cell kinetics, hormone production mechanisms, RID and RIT.

There have been many attempts to devise xenograft systems in the past but the results were disappointing and had numerous setbacks. The initial use of immunologically privileged sites for xenografting—e.g. the anterior chamber of the eye, the brain, the hamster cheek pouch and others—did not fulfil early expectations. With a fuller knowledge of the biology of tumour graft rejection mediated through cellular immunity (T-lymphocytes)[4], it became feasible to immune-suppress the recipient animals usually mice in various ways right from the use of whole body irradiation supplemented with corticosteroids and other immune-suppressive drugs reported to be extremely toxic for the host animal[5] or thymectomy at 3–4 weeks of age and treatment by antilymphocytic sera (ALS)/antithymocytic sera (ATS)[6–9] together with marrow reconstitution to combat high radiation dose and use of marrow rescuers e.g. Ara-C[10–12]. Several human tumours were successfully established and propagated as xenografts in these systems though certain technical problems were encountered including the disadvantages arising from reconstitution by syngeneic bone marrow which contains T cells or their precursors.

Such problems appeared to have become redundant, however with the introduction of genetically immune-suppressed recipients principally the athymic nude mice. These animals lack the capacity to reject foreign tumour grafts to a degree which compares favourably with the most profoundly immune-suppressed animals by any of the previous methods. However, these did not prove ideal for laboratories in India because their maintenance is not economic; further they soon become diseased altering the drug response[13]; not all tumours grow in them specially those possessing tumour rejection antigen (TRA)[14] and metastases are rare probably due to high natural killer (NK) cells level[15].

Although thymectomy under general anaesthesia sounds essential for better immune-suppression, it is not always easy in new hands and that too on only 3–4 weeks old mice. Secondly, T-deprived mice may recover their immune competence after 6–8 weeks of irradiation. Use of ALS and ATS antisera is limited by availability and cost etc. We therefore thought it necessary to maintain immune-suppression with a titrated dose of corticosteroids which is neither lethal to the graft nor to the host and yet does not carry a significantly enhanced infection rate making the protocol quite economic.

It has been reported that some tumours e.g. malignant melanomas, colorectal adenocarcinoma,

pancreatic carcinomas, bronchial carcinomas etc. are relatively easy to establish as xenografts but lymphomas, leukaemias, prostatic and breast malignancies have proved to be difficult[11, 16, 17]. The present report describes our attempts to grow specially human breast tumours as xenografts after preliminary *in vitro* storage as this is the second most commonest female malignancy[18] and also because these tissues are easily available in a relatively sterile state and thus the model may prove to be quite useful in our future RID and/or RIT programme. Carcinogen or virus-induced tumours in animals are not suitable for our above programme because the antigenic composition of human tumours is quite different from that of induced ones.

RID has been achieved by the use of specific monoclonal antibodies which conferred a possible third dimension of etiopathological imaging to conventional anatomical and physiological imaging delineating structure and function respectively[1]. The concept of attaching radionuclides to antisera is quite old but the development of hybridoma technology has transformed the picture by enabling the *in vitro* production of unlimited quantities of precise well-defined immunoglobulin species[19]. Apart from mouse hybridomas, human hybridomas and direct transformation of immunized lymphocytes either from animals or from the patient himself have also been utilized to make monoclonal antibodies[2]. The tumour antigens earlier studied were the shed antigens such as CEA or β-hCG. It was surprising that worthwhile pictures were achieved with antibody directed against these despite mopping up in the circulation. There is now greater interest in cell-fixed antigens such as the milk fat globule[20], and an antibody directed against these was used for imaging the breast carcinoma xenografts.

MATERIAL AND METHODS

Immune suppression was achieved in Swiss albino mice weighing 20–25 g by 7 Gy whole body irradiation with a ^{60}Co teletherapy unit and was maintained in the post-xenografting period by systemic steroids[21] (bethamethasone 0.8 mg administered by intramuscular parenteral route on alternate days). The animals were kept in a conventional animal house rather than the ultraaseptic facilities used in maintaining nude mice. Chances of infection were minimized by giving co-trimaxozole.

Fresh tumour pieces were collected in a cold transport medium containing Hank's balanced salt solution supplemented with antibiotics (penicillin, streptomycin, etc.) and carried to the laboratory with a minimum delay where part of the tissue was processed for histopathology and immunoperoxidase staining. The rest was cleaned of any necrotic and fatty material, rewashed with Dulbeco's balanced salt solution and stored at 37°C in Minimum Essential Medium (MEM) or Rosewell Park Memorial Institute (RPMI) medium supplemented with 5% fetal calf serum for at least 120 h to help eliminate tumour lymphocytes and other factors contributing to graft rejection.

Small 4–6 mm healthy cubes of the malignant tissue were implanted subcutaneously[13] through incisions usually at the interscapular or left shoulder region, sites which are far removed from the liver and spleen (non-specific sites of radiolabelled antibody accumulation when scanning is performed) so as to avoid superimposition of the target and non-target areas in the scan. Local antibiotic (Neosporin) was instilled. The wound was closed by 3–0 catgut and sealed by a polyvinyl polymer (Healex) spray.

Radio-iodination

Radio-iodination of the monoclonal antibody HMFG-2 was performed with ^{125}I (IMS 30-Amersham) using strict aseptic solid phase lactoperoxidase technique[22]. The radio-pharmaceutical diluted in 0.9% w/v sodium chloride was filtered through a Millex-GV 0.22 μm filter unit and injected intravenously in a volume of 0.5 ml/mouse with an estimated activity of ~ 10 MBq after prior block of the thyroid by oral iodine 1 mg/day for five days. In a control group iodinated non-immune globulin was injected.

Radio-immunoimaging was performed 1–4 weeks after the administration of radio-pharmaceutical and repeated after another 10 days. Imaging was performed with a Siemens ZL 7500 gamma camera using a pin-hole collimator after peaking the system with ^{125}I.

RESULTS

Tumour take has been noticed as early as on the 11th day with new vascularization without any sign of inflammation. Longest survival of the breast tumour xenografted was for 104 days (figure 1). A mouse who survived for 64 days developed metastatic spread in the left axilla and secondary lymphoedema (figure 2). Reticuloendothelial system showed giant cell reaction with the presence of

Figure 1. Mouse showing xenografted human breast carcinoma.

plasma cells suggesting the host graft rejection response.

On imaging, the control group in whom non-immune globulin was injected showed no specific localization, while the breast tumour xenografted mice receiving iodinated anti-HMFG-2 monoclonal antibody showed a significant accumulation at the target site with a comparatively much lower uptake by the liver. This non-specific accumulation had been further cleared in ten days later second scan (figure 3). No other site of non-specific accumulation was observed.

Successful breast tumour xenograft model and radiolocalization using radiolabelled specific monoclonal antibodies has potential advantage over almost all current imaging techniques specially for lung and liver lesions where infective, primary and metastatic lesions are all common and confusing. The 1–4 week

Figure 2. Oedamatous left upper limb of the mouse with metastasis.

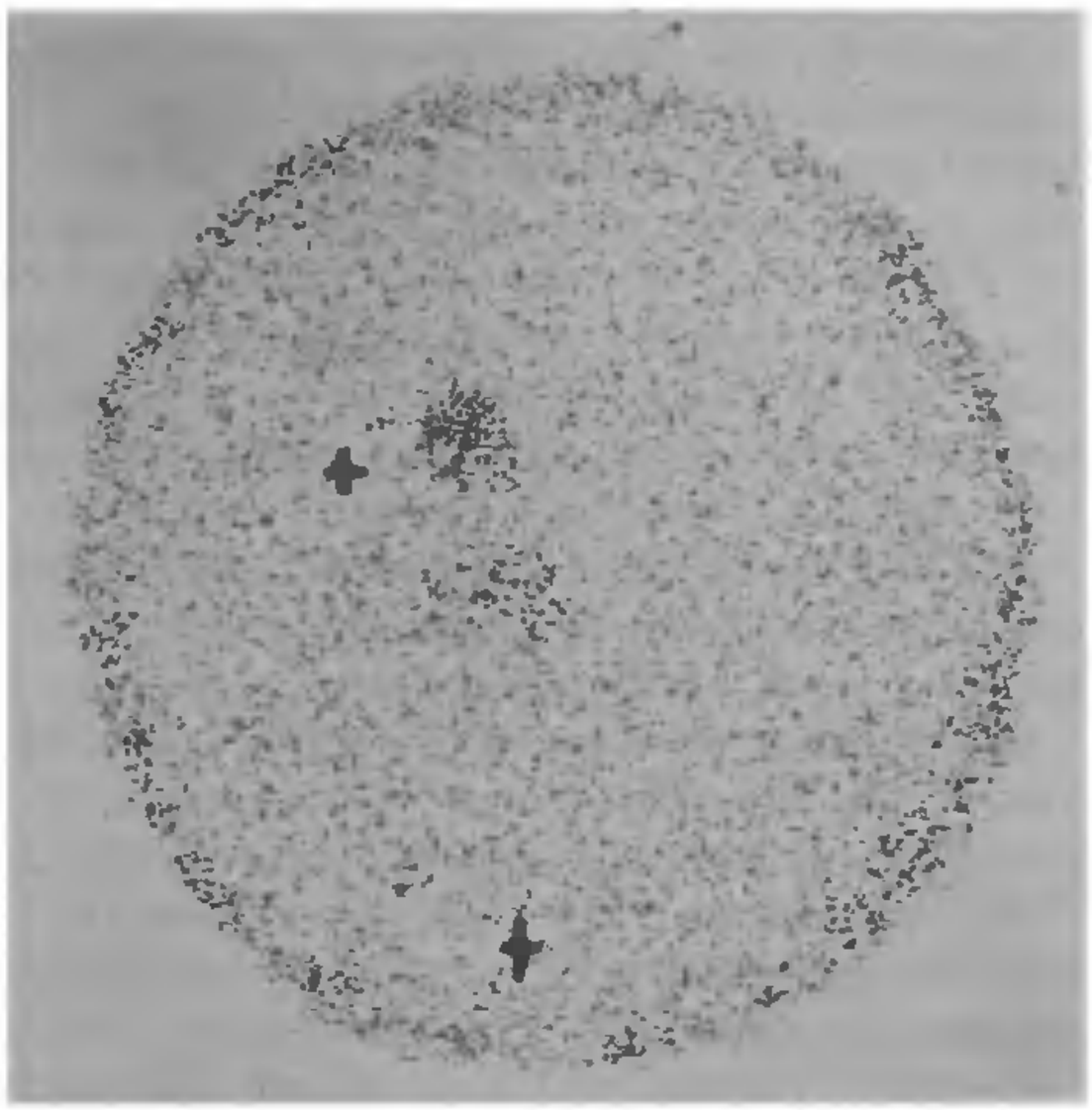

Figure 3. Radioimmunotargetting of the xenograft showing minimum trapping by the liver.

period allowed between the radiopharmaceutical injection and first scanning permitted the accumulation of the radiolabelled antibody in the target lesion and the clearance of free radio-iodine from the non-target areas. Both this initial period as well as the gap between the first and the second scan also facilitated the clearance of non-specifically localized radiolabelled antibody especially from the reticuloendothelial system (RES) as evidenced by the significantly reduced liver uptake in the second scan.

Similar studies with other monoclonal antibodies and further specificity studies have now been undertaken. The successful creation of breast carcinoma xenografts has been very encouraging. Apart from RID and/or RIT this opens the field for trial of chemotherapy or endocrine therapy, assessment of the malignant potential of human tumour cells, and biological studies of human tumour xenografts for metastasizing behaviour, biochemistry, hormone production viral status, etc.

Modifications for future clinical use that are under study are evaluation of different isotopes, chromatographic purification of the radiolabelled antibody and performance of the scan 2–5 days after radiopharmaceutical injection.

An ideal radionuclide label[23] suitable for our RID programme will be based on: (i) pure gamma emission with energy suitable for external detection by scintillation cameras or scanners, and (ii) half-life long enough to permit visualization after clearing off blood or RES background i.e. non-target

radioactivity and also permitting radiochemical manipulation for linkage to the vehicle (if any), but short enough to permit sufficient radiopharmaceutical administration for pictures of adequate information density without unacceptable radiation dose. Other desirable features include minimal complexity of photon spectrum, easy accessibility, low cost of production and radiochemical purity for biodistribution. Dewey and Sinclair[24] have mathematically defined desired radionuclidic target versus non-target concentrations to permit adequate imaging for a given detection system.

The requirements of isotope for RIT are different from those of RID and have been discussed[25]. Possible isotopes for use in India suggested include ^{199}Au, ^{111}Ag and ^{143}Pr-radionuclides[26] and their economic production is currently being explored in collaboration with BARC, Bombay, India.

Successful radioimmunotargetting in RID also heralds a chapter of selective RIT. Considering various warheads that can be attached to a homing monoclonal antibody, radioisotope seems to be superior to a cytochemical warhead in the sense that it is not cycle-dependent, gives no time and chance to the target cells to bypass the lethal effect[27], and can kill bystander tumour cells (in a 30 cell diameter zone) even if they have not taken up the labelled antibody, unlike the requirements for immunotoxin and cytochemical warheads of entry into *all* cancerous cells[28].

ACKNOWLEDGEMENTS

The authors are grateful for the generous gifts of anti-HMFG-2 monoclonal antibodies from Dr J. T. Papadimitrou and Associates, ICRF, London, UK and to Maj. Gen. N. Lakshmipathi and Dr G. Narayanan, Institute of Nuclear Medicine and Allied Sciences, Delhi, for gamma camera imaging. The authors are also grateful to Drs B. R. Elhence, K. Singh, R. N. L. Srivastava, B. Arvind, S. Khandelwal, V. K. Rohtagi, R. Singh and Mrs M. Kumari, S.N. Medical College, Agra, India, who are the other important members engaged in this work. This work was supported by a grant from DST, New Delhi.

22 June 1987

1. Hazra, D. K. *et al.*, *Nucl. Med. Commun.*, 1987, **8**, 139.
2. Saran, S., Lahiri, V. L., Hazra, D. K. and Singh, R., *Curr. Sci.*, 1985, **54**, 1036.
3. Handler, A. H., *Fedn. Proc. Am. Soc. Exp. Biol.*, 1956, **15**, 517.
4. Davies, A. J. S., In: *Immunodeficient animals for cancer research*, (ed.) Stephen Sparrow, Macmillan, London, 1980, p. 2.
5. Giovanella, B. C., *Oncology supplement, scientific foundation of oncology*, (eds) T. Symington and R. L. Carter, Won Heinmann Med. Books, London, 1980.
6. Castro, J. E., *Nature* (*New Biol.*), 1972, **239**, 83.
7. Cobb, L. M. and Mitchley, B. C. V., *Eur. J. Cancer*, 1974, **10**, 473.
8. Berenbaum, M. C., Sheard, C. E., Reittie, J. R. and Bundick, R. V., *Br. J. Cancer*, 1974, **30**, 13.
9. Detre, S. I. and Guzet, J. C., *Br. J. Cancer*, 1973, **28**, 412.
10. Miller, J. L., Hudspith, B. N. and Blackett, N. M., *Br. J. Cancer*, 1975, **32**, 193.
11. Miller, J. L., *Exp. Haematol. Suppl.*, 1976, 68 (abstract).
12. Steel, G. G., Courtenay, V. D. and Hostom, A. Y., *Br. J. Cancer*, 1978. **37**, 224.
13. Povlsen, C. O., Spang-Thomsen, M., Rygaard, J. and Visfeldt, J., In: *Immunodeficient animals for cancer research*, (ed.) Stephen Sparrow, Macmillan, London, 1980, p. 95.
14. Moore, M., In: *Immunodeficient animals for cancer research*, (ed.) Stephen Sparrow, Macmillan, London, 1980, p. 135.
15. Festing, M. F. W., In: *Immunodeficient animals for cancer research*, (ed.) Stephen Sparrow, Macmillan, London, 1980, p. 17.
16. Detre, S. I., Davies, A. J. S. and Connors, T. A., *Cancer Chemother. Rep.*, 1975, **5**, 133.
17. Shimosato, Y. *et al.*, *J. Natl. Cancer Inst.*, 1976, **56**, 1251.
18. Saran, S. and Agarwal, G. N., *Indian J. Cancer*, 1984–85, **21**, 133.
19. Kohler, G. and Milstein, C., *Nature* (*London*), 1975, **256**, 495.
20. Epenetos, A. A. *et al.*, *Lancet*, 1982, **2**, 999.
21. Toolan, H. W., *Proc. Am. Cancer Res.*, 1953, **1**, 56.
22. Murphy, M. J. Jr., *Biochem. J.*, 1976, **159**, 287.
23. Hazra, D. K. and Dass, S., *Nucl. Med. Commun.*, 1983, **4**, 40.
24. Dewey, W. C. and Sinclair, W. K., *Indian J. Appl. Radiat. Isot.*, 1961, **10**, 1.
25. Hazra, D. K. *et al.*, *Advances in the applications of monoclonal antibodies in clinical oncology*, Royal Postgraduate Medical School, London, May 1985.

26. Hazra, D. K. *et al.*, *Br. J. Cancer*, 1986, **54,** 550.
27. Panel proceedings series on tumour localization with radioactive agents, IAEA, Vienna, 1976, p. 138.
28. Hazra, D. K., Shukla, A. K. and Saran, S., *S.N.M. Newsl.*, 1984, **8,** 3.

ANNOUNCEMENT

NATIONAL WORKSHOP ON MODIFICATION OF MATERIALS BY ION BEAMS

This Workshop will be held at the Department of Physics, University of Bombay, Bombay, from February 25 to 26, 1988. The workshop aims at making the participants well acquainted with the present state of art in this field in the country. It will also provide a forum to exchange views and expertise through invited talks, paper presentations and discussions. The workshop will be dealing with the following topics: 1. Ion-implantation in metals, semiconductors, insulators, polymers, etc.; 2. Hard coatings using ion beams; 3. Device fabrication; 4. Radiation damage; 5. Ion beam interaction with matter, and 6. Ion beam technology.

Further particulars may be had from: Prof. R. Pratap, Convener, National Workshop on "Modification of Materials by Ion Beams", Department of Physics, University of Bombay, Vidyanagari, Santacruz (East), Bombay 400 098.