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RADIOIMMUNODETECTION OF MALIGNANCIES USING ANTIBODIES TO PRIMARY AND SECONDARY TUMOUR ANTIGENS

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The Concepι of using autologous or heterologous antibodies in the management of cancer has fascinated researchers for more than 100 years, and it was a challenge to nuclear medicine when in the mid-fifties the use of radiolabelled antibodies for tumour detection was suggested. During the early period of the approaches to immunoscintigraphy, however, only little success has been achieved in obtaining sufficiently purified tumour-specific antibodies, and it was only recently that considerable progress was made. Interest was focused at first on the possibility of using aberrant molecules as antigens which are located on the surface of malignant cells, and in fact there have been discovered in recent years a series of such structures in different types of tumour, i.g. melanomas, osteosarcomas, ovarian tumours, and Hodgkin's disease. Like researchers in the U.S.A. and in various European countries we have used antibodies of well-defined specificity directed against a glycoprotein component of the cell membrane of melanomas for scintigraphic imaging of such tumours and their metastases.

More commonly, tumour-associated antigens are used in animal experiments as well as in clinical practice, such as carcinoembryonic antigen, labelled antibodies which have been shown by serveral groups to localize in various carcinomas. In view of the fact, however, that the uptake in the tumour region, when compared to adjacent areas, is only small in most cases, image processing by background subtracting is frequently necessary for distinct tumour imaging. By this means we were able to demonstrate scintigraphically malignant growth, i.g. a teratocarcinoma with β hCG.

In spite of an increasing number of similar results attained by researchers and clinicians all over the world, a number of questions remain unanswered which are of great significance for the further development of radioimmunodetection. Thus, for example, 25 years ago we had already become aware of a problem when we tried on the bases of experiments of David Pressmann, to produce tumour-specific antibodies by immunization of rabbits with homogenates of Yoshida sarcomas. We were in fact able to achieve antibodies which after labelling with radioiodine and injecting into the tumour-bearing animals showed a marked uptake in the tumours. However, it was later revealed that the antigen against which our antibodies were directed was fibrin. And fibrin-clotting can, of course, occur not only in tumours but also in inflammatory processes. Although in our case it was in fact an antigen-antibody-reaction, at least the antigen was unspecific for malignancies. Moreover, in the following years it was ascertained that tumours can pick up a

great amount of circulating foreign substances, due to altered structures in the stroma vessels, increased perfusion, pinocytosis, or for other yet unknown reasons.

We have therefore concentreated our interest on the problem of specifity and in close cooperation with Bjorklund in Stockholm we have investigated the mechanisms which are responsible for the accumulation of antibodies against tissue polypetide antigen in different tumours. In a number of our experiments we have used ACI-rats in which tumours had been implanted. In the course of these investigations it was, for example, possible to descern a marked TPA-ab uptake in a xenograft of rat urothelial carcinoma. The antibodies had been produced by immunization of a horse and we considered the possibility that horse TPA-ab could have been unspecifically trapped in the tumour. To study this possibility we gave the tumour-bearing rats injections of normal horse IgG as well as of the usual anti-TPA-ab, and as a result we found tumour uptake of the labelled proteins in both cases. The quantitative analysis, however, revealed that the target- to non-target-ratio was somewhat higher for the TPA-specific antibodies. In a subsequent experiment the animals were at first injected with unspecific and non-radioactive labelled IgG. Later-on the application of labelled anti-TPA-ab as well as of labelled IgG was carried out. We found that only little uptake occurred in the case of labelled unspecific immunoglobulines in the tumour, whereas an anti-TPA-fixation was distinctly ascertained.

Because of the relationship between TPA and components of the cytoskeleton we were interested in the question of whether the antibody binding could be demonstrated microscopically and in what kind of cells this is possible. In these experiments we used for the labelling of the TPA-ab the fluorescent dye FITC, and UV-irradiation revealed the existence of TPA in a network of fine filaments in Hela-cells during the interphase, whereas during the metaphase TPA is aggregated mainly at the cell surface. As an interesting finding it should be mentioned that TPA was exclusively found in epithelial cells, but not in cells of mesenchymal origin. Thus, anti-TPA-ab are not fixed in sarcoma tissues, a fact which could impressively be demonstrated in PAP-dyed histological sections of a carcinosarcoma of the urinary bladder. Only the carcinomatous portion of the tumour gave histochemical proof of TPA-ab binding.

The observation that TPA occurs only in epithelial tissues and not in sarcomas seems to be contradicted by the fact that TPA is also found in the circulation of sarcoma patients. The explanation for this might be that inflammatory changes are always present in the environment of malignant tumours, and this to a more marked extent the quicker the tumour grows. This, however, leads to the destruction of normal tissues, and it can be assumed that increased TPA levels in sarcoma-bearing patients are based on this mechanism. -

We have hitherto dealt with tumour antigens which have to be considered either as specific constituents of the cell membrane or as naturally occurring tumour-associated antigens. As a new potential for immunoimaging of tumours we have looked into a different possibility in experimental approaches. The aim of these studies was to introduce artificially antigenic material into tumours and to produce antibodies directed against it which can be used for scintiimaging. For this purpose certain microorganisms appeared to be suitable, i.g. oncolytic vira or tumour-specific bacteria.

In earlier publications we have already reported that anaerobic spores, when injected intravenously into a tumour-bearing animal, germinate selectively in malignant tissues but not in normal organs. This was based on former observations of Malmgren et al. according to which the

vegetative form of Clostridium tetani is localized in mouse tumours following i.v. spore administration. We have used the apathogenic Clostridium butyricum for our experiments the behaviour of which is similar to that of Cl. tetani. The anaerobic milieu in the cancerous tissue is assumed to be an important reason for the tumour-selective germination.

Antibodies against the bacteria were raised in rabbits and after purification and radioactive labelling injected into tumour-rats which were pre-treated with spores. As a result, we found a marked radioacitivity uptake in the tumour when there were vegetative forms of Clostridium butyricum present. Through FITC-labelling of the antibodies the immune complexes could be demonstrated by fluorescent microscopy. Gram-staining of tissue slices of various organs of the spore-treated animals gave no evidence of the germination of bacteria in healthy tissues, as it was expected.

We have not yet used antibody fragments, but these investigations are to begin shortly and we hope that it will thus be possible to achieve a still greater activity accumulation in tumours. Furthermore, the production of monoclonal antibodies is planned which will possibly lead to a further improvement.

In view of a clinical application of the new method it would naturally be necessary to clarify a number of questions. As, however, the used Clostridia strain is apathogenic, and extra-tumoral germination can be ruled out in all probability, it seems, in our opinion, worth-while to pursue the immunoscintigraphic studies with tumour-residing microorganisms which we have named "secondary tumour antigens".

In summing up it can be stated that there are still many problems with radio-immunodetection of cancer, but that evidence indicates that present experimental and clinical approaches will soon lead to valid solutions.