less invasive techniques like focused ultrasound therapy for renal tumors, bladder tumors and prostatic carcinomas are rapidly evolving.

These new techniques in urologic oncology and other noninvasive techniques that have been developed in the last years have a tremendous impact on urology and will eventually change this medical speciality.

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REJECTION OF CYTOKINE GENE TRANSFECTED MOUSE TUMORS: THERAPEUTICAL IMPLICATIONS

Th. Blankenstein
Max-Delbrück-Center for Molecular Medicine, 13122 Berlin, Germany

Cytokines provided locally at the tumor site may initiate an effective anti-tumor immune response which leads to rejection of a tumor which otherwise grows progressively. Experimentally, this can be tested by gene transfer into cultured tumor cells followed by the analysis of the tumorigenicity of such genetically engineered cells. This approach allows to analyse the function of a given cytokine *in vivo* and to elucidate the therapeutical value of genetically engineered tumor cells as vaccines. Our experience includes experiments with about ten cytokines and the results can be summarized as follows: (1) some cytokines possess anti-tumor activity in this system, others do not; (2) a local and continuous cytokine supply seems to be essential for tumor rejection; (3) the tumor cell derived cytokines act in a dose-dependent manner and in the absence of systemic toxicity; (4) the immunological effector mechanisms induced by different cytokines are partly cytokine-specific, partly redundant (and usually involve T cell dependent and independent mechanisms; (5) tumor rejection and mechanism thereof may be different with different tumor cell lines transfected with the same cytokine gene.

Cytokine gene modified tumor cells as vaccines are currently tested in first clinical trials. However, critical parameters such as vaccine potency of cytokine gene transfected tumor cells, optimal level of cytokine expression, reasons for varying vaccine effects in different tumor models, influence of irradiation of vaccine cells on their efficacy and attempts to improve vaccine efficacy (e.g. by coexpression of cytokines and T cell costimulatory molecules as B7) have to be further addressed in experimental tumor models.

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PRECLINICAL MODEL FOR GENE THERAPY: ROLE OF THE HOST

M. P. Colombo[1], G. Forni[2], A. Stoppacciaro[3]
[1]Istituto Nazionale Tumori, Milan
[2]CNR Centre of Immunogenetics and Oncology, Turin
[3]Department of Biopathology, University of Rome, Italy

Three different experimental systems based on cytokine gene transduction can provide evidence that (I) systemic immunity does not always follow tumor regression; (II) a cytokine combination that efficiently induces systemic immunity does not induce CTL activity and does not exert therapeutic effects; (III) a different cytokine combination induces both CTL and protection in Winn assay without inhibiting tumor take and outgrowth.

(I) C-26/G-CSF regression is coupled with infiltration of leukocytes releasing secondary cytokines and depends on $CD8^+$T cells, regressor mice however, remain susceptible to a challenge with C-26 cells.

(II) TSA/IL-4 more efficiently than TSA/IL-2 induce protection against a challenge with live TSA cells. To transfer IL-4 mediated systemic immunity, both lymphocytes and serum from immune mice are needed. TSA/IL-4 cells when used as vaccine to cure TSA bearing mice were without effect, whereas TSA/IL-2 were moderately effective.

(III) C-26/IL-12 cells showed delayed tumor onset that was NK dependent. Immunocytochemical characterisation of leukocytes infiltrating C-26/IL-12 tumors showed few infiltrating T cells in non-depleted mice but abundant infiltration by $CD8^+$ T cells in tumors from mice depleted of $CD4^+$ T cells and CD4 depletion allowed tumor regression in about 30% of mice. This is not due to a CD4-mediated suppression since mice primed with C-26/IL-12 cells possessed lytic lymphocytes and $CD8^+$ T cell which mediated protection against C-26/IL-12 in a Winn assay.

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GENE AND PEPTIDE THERAPY OF TUMOR METASTASES IN MURINE MODELS

L. Eisenbach, O. Mandelboim, A. Porgador, D. Plaksin, M. Fridkin

Highly metastatic clones of malignant murine tumors are characterized by low immunogenicity and reduced MHC Class I expression. Metastatic lesions of human tumors are similarly impaired in HLA expression. Gene modification using plasmid and retroviral vectors carrying cDNAs for MHC Class I, γIFN, IL-2 or IL-6 increases immunogenicity of lung carcinoma (3LL) and melanoma (B16) cells. Vaccines based on irradiated gene modified cells and their combinations were used in diseased animals and achieved certain cure rates. CTL recognize peptide sequences of defined length presented in the groove of MHC class I. TAA peptides presented by H-$2K^b$ were purified from 3LL carcinoma and proven to be mutants of a peptide from the gap junction protein connexin 37 and normal peptides of an aberrantly expressed β globin gene. Structural aspects and therapeutic efficacy of peptide vaccines will be discussed.

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COMBINATION GENE THERAPIES FOR THE TREATMENT OF MALIGNANT MELANOMA *IN VIVO*

R. Vilea, Heung Chong, R. M. Diaz[1], I. Hart[1], S. Castleden, A. Tuszynski
Imperial Cancer Research Fund Laboratory of Cancer Gene Therapy
[1]Richard Dimbleby Cancer Research Laboratory, Rayne Institute, St Thomas Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

For *in vivo* gene delivery, we have used the murine tyrosinase promoter to restrict expression of genes to melanoma cells. These genes are aimed either at enhancing the immunogenicity of tumour cells (cytokines and members of the B7 family of genes) or at killing tumour cells directly (HSV*tk*). Recently, we have observed, and characterised, the generation of an anti tumour immune response following *in vivo* killing of established tumour deposits with HSV*tk*, suggesting that both approaches can be combined to improve the efficacy of *in vivo* gene therapy. Data will be presented on the development of novel double expression vectors in which the HSV*tk* gene is co-expressed with a series of immunomodulatory genes to augment this anti-tumour immunity. Our data demonstrate that protocols aimed at enhancing tumour cell immunogenicity *in vivo* are most likely to be successful by the co-expression of more than just a single therapeutic gene within the tumour cells.

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MUCINS AS MARKERS OF CELL DIFFERENTIATION AND NEOPLASTIC TRANSFORMATION

F. X. Real
Institut Municipal d'Investigació Mèdica, 08003-Barcelona, Spain

Mucins are synthesized by glandular epithelia and are the major components of mucus. The cloning of cDNAs encoding human apomucins facilitates the analysis of their structural complexity and heterogeneity. Until now, 8 independent genes (MUC1-MUC7) have been identified which encode Ser/Thr-rich proteins with repetitive domains.

Using these cDNAs, as well as anti-apomucin antibodies raised against a variety of immunogens (i.e. native and deglycosylated mucins, synthetic peptides, fusion proteins), a considerable amount of information has been obtained regarding the pattern of expression of each gene in tissues. Each mucin gene has a distinct normal tissue distribution. Thus, MUC1 and MUC5B are expressed in a wide variety of normal epithelia, whereas MUC2 is mainly expressed in the intestine, MUC5AC in the respiratory tract and in the stomach, and MUC6 in the antrum. Multiple mucin genes can be expressed in a given tissue and at the single cell level, although in certain tissues a high degree of specialization is observed: in the stomach MUC5AC is present in the superficial epithelium whereas MUC6 is present in antral glands. In the stomach, apomucin expression correlates with Lewis antigen expression, although it is not clear whether the primary amino acid sequence of mucins contains instructive signals for glycosylation.

Altered expression of mucin genes in pathologic states has now been demonstrated, in particular in cancer tissues. In colonic and gastric cancers, loss of expression of MUC2 and MUC5AC takes place, respectively. In contrast, MUC2, MUC4 and MUC5AC are aberrantly expressed in pancreas cancer tissues. In benign proliferative lesions of the colon and the pancreas, changes in the expression of mucin genes have also been demonstrated. Preliminary data suggest that the pattern of mucin gene expression in cancer tissues may be related to the biological behaviour, although more work is necessary in this area.

Cultured cell lines have been used to study the regulation of mucin gene expression *in vitro* facilitating the study of the biology of mucin-producing cells.

In conclusion, it is now possible to establish the mucin phenotype of tumors at the molecular level. This information allows a better study of the alterations of cell differentiation occurring during neoplastic transformation as well as the role of mucins in tumor progression.

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CELL SURFACE BOUND MUCINS AND TUMOR PROGRESSION

J. Hilkens, J. Wesseling, H.L. Vos, C. Patriarca, M. Boer, J. Storm, S. van der Valk, J. Calafat

Division of Tumor Biology, The Netherlands Cancer Institute (Antoni van Leeuwenhoekhuis), Plesmanlaan 121, 1066 CX Amsterdam, The Netherlands

We have investigated the role of episialin, a surface bound mucin also known as EMA, PEM, CA 15-3 etc. encoded by the MUC1 gene, in tumor progression. The molecule has an extended rod-like structure protruding more than 200 nm above the plasma membrane. The expression of the molecule is usually more than ten times that in normal epithelia as determined by *in situ* hybridization and can be extremely high on metastatic cells present in pleural effusions. Episialin overexpression strongly reduces cell-cell and cell-matrix adhesion. The anti-adhesion properties of the molecule are due to the extreme length of the molecule since genetically modified molecules with a reduced length did not exhibit the anti-adhesion effect. E-cadherin/episialin double transfectants showed that episialin can prevent E-cadherin mediated cell-cell adhesion. Decreased E-cadherin mediated cell-cell interactions are known to promote invasion. Episialin overexpression is expected to have the same effect. Indeed, episialin overexpression promoted invasion in matrigel. Episialin overexpression at the cell-stroma boundary in primary breast cancers caused large "clefts" between the stroma and tumor cells. These results suggest that episialin has the same anti-adhesion properties *in vivo*. Episialin also interfered with immune recognition. Melanoma transfectants expressing high levels of episialin were less susceptible to lysis by LAK cells and allogeneically stimulated T-lymphocytes. The same transfectants had a significantly higher propensity to form lung metastases after i.v. injection in nude mice than episialin negative revertants of the same clones. Episialin may protect the tumor cells against NK cells and/or episialin expressing cells are more likely to metastasize as a result of the decreased cell-cell interactions. Our results strongly suggest that episialin overexpression is an important factor in tumor progression.

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IMMUNE RESPONSE TO THE POLYMORPHIC EPITHELIAL MUCIN (PEM)

M. Nuti, A. Rughetti, C. Petrarca, H. Rahimi, F. D'Agostini, C. Apollonj Ghetti, G. Scambia, L. Frati

Department of Experimental Medicine, University of Rome, Italy

Department of Biomedicine, University of Pisa, Italy

Department of Gynecology, Catholic University of Rome, Italy

Several tumor associated antigens have been shown to be able to induce an immune response. The identification of such antigens and the effector mechanisms involved is a first step for the development of useful cancer vaccines. PEM is a glycoprotein localized on the luminal surface of most simple epithelia. In cancer cells it is overexpressed and undergoes a process of aberrant glycosylation. Cryptic epitopes within the core protein of the extracellular domain are therefore exposed and could be a target for an immune response. An MHC unrestricted T cell response mediated by CD8 cells was described and explained by the particular structure of the molecule made up of tandem repeats (TR). Lymphocytes from tumor draining lymph nodes from patients with gynecological malignancies were utilized to study both the humoral and cellular immune responses of these patients. The human antibodies produced by these patients were directed against different epitopes within the TR sequence of PEM and were able to recognize the tumor associated glycoforms of the molecule. T cell clones were isolated that were able to proliferate in the presence of specific peptides corresponding to the TR of PEM presented by autologous B cells. The functional analysis of these clones revealed a Th phenotype. The possible contribution of T helper cells in generating and maintaining anti-tumor immunity opens new possibilities for effective immunological approaches in cancer therapy.

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THE MUC1 GENE PRODUCT, PEM AS A TARGET ANTIGEN IN CARCINOMAS

J. Taylor-Papadimitriou, J.M. Burchell, R. Graham

Imperial Cancer Research Fund, 44 Lincoln's Inn Fields, London WC2A 3PX, U.K.

Most glandular epithelial cells lining ducts express on their apical surface a transmembrane glycoprotein the polymorphic epithelial mucin (PEM) which is heavily O-glycosylated. PEM is overexpressed in more than 90% of breast and ovarian carcinomas and in some lung and colon tumours. Although the sequence of the MUC1 gene coding for the core protein is not altered in the cancer associated mucin, the O-glycans which are added are shorter and more heavily sialylated. This results in the appearance of novel carbohydrate epitopes and the unmasking of core protein epitopes which lie between the O-glycosylation sites. The increased expression of an antigenically distinct molecule, which is no longer restricted in its expression to the apical surface appears to induce both B and T cell responses in some breast, ovarian and pancreatic cancer patients.

To compare immunogens based on the MUC1 gene or its product, and to optimise antigen presentation, syngeneic and transgenic mouse models have been developed. In the syngeneic model, intramuscular injection of cDNA coding for the MUC1 gene (driven by the actin promoter) given before injection of MUC1-expressing mouse tumour cells can inhibit tumour growth. In the animals rejecting the tumour, cytotoxic T cells can be isolated and their development correlates to some degree with effective tumor immunity, while the appearance of circulating antibodies does not. In a mouse transgenic for the human MUC1 gene, PEM expressing tumours are rejected when antigen presentation is enhanced by expression of the B7 molecule.