(HT-1080) were used as a model to study the effects of TGF-β on the cell-secreted proteolytic activity and deposition of extracellular proteins to the growth substratum. The secretion of plasminogen activators (u-PA and t-PA) and the endothelial type plasminogen activator inhibitor (PAI-1) were quantitated using caseinolysis assays, zymography and reverse zymography. TFG-β caused a significant decrease in the amounts of secreted u-PA and t-PA in WI-38 and CCL-137 cell lines. Concomitantly, the enhanced secretion and deposition of PAI-1 was observed both in WI-38 and HT-1080 cell lines. The deposition of PAI-1 was a primary effect of TGF-β and occurred rapidly within 8 hr. The accumulation of PAI-1 to the medium was more slowly as shown by metabolic labelings and pulse-chase experiments. The deposited PAI-1 was sensitive to removal by u-PA. Subsequently, complexes of higher molecular weight were detected in the medium. Our results suggest that a rapid and sensitive effect of TGF-β on both normal fibroblasts and malignant cells is the reduction of proteolytic activity which may be associated with the growth inhibitory properties of TGF-β.

ACTIVATION OF PROTEIN KINASE C IN INTACT HUMAN PLATELETS BY ANTHRACYCLINE-IRON COMPLEXES

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Doxorubicin activates human platelets while daunorubicin inhibits both serotonin release and protein kinase C (PKC) activation in thrombin. Complexation with Fe (III) decreased the concentration of Doxorubicin necessary to induce platelet activation and reversed the effect of daunorubicin from inhibition to activation of PKC. N-acetyl-doxorubicin remained ineffective even in the presence of Fe. Addition of catalase or superoxide dismutase had no effect on the activation; nevertheless the determination of malondialdehyde by the thiobarbituric acid method showed an increse of lipid peroxidation in platelets treated with the iron complexes that followed the same pattern of the activation of PKC. These results suggest that PKC activation in doxorubicin treated platelets could be mediated by free radical formation and lipid peroxidation.

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PHENOTYPE OF METASTATIC CELLS AS TARGET FOR ANTI-METASTATIC INTERVENTIONS

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The surface of highly metastatic Lewis tumour was characterised by: (1)increased GAG biosynthesis, (2)increased heparan sulphate/chondroitin sulphate ratio, (3)increased sialylation of gal/galNac terminated glycoproteins. As a consequence of membrane properties, the highly metastatic cells expressed high affinity to ECM components (GAG), fibronectin, collagen I-III, and showed immunoresistance against NK cells and macrophages. Meanwhile there was no change in cell proliferation kinetics. Targets for anti-metastatic interventions were as follows: (1)proliferation (CY.13324, tiazofurin), (2)cell membrane (KL-1o3; anti-GAG agent), (3)heterotypic interactions (PGI_2) (4)immunoresistance (KL-1o3, lentinan, macrophage infusion). The anti-proliferative agents were equally effective against tumour lines. The anti-GAG agent - immunotherapy - was able to inhibit the highly metastatic tumour, probably altering the heterotypic interactions and turning the immunoresistant tumour immunosensitive again. The PGI_2 and macrophage infusion proved to be effective only against immunosensitive tumours.

GROWTH FRACTION/DNA ANALYSIS USING Ki-67 ANTIBODY IN FLOW CYTOMETRY

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The nuclear antigen Ki-67 present in proliferating cells (Gerdes et al, J. Immunol., 133: 1710, 1984) was determined flow cytometrically in PHA stimulated lymphocytes and in HL-60 human promyelocyte leukaemia cells. In stimulated lymphocytes 81% of cells were found to be Ki-67 positive in comparison to 85% positive with anti-bromodeoxyuridine and 80% and 77% positive with the antibody independent staining methods using Hoechst 33342/ethidium bromide and mithramycin, respectiely. In HL-60 cells induced to differentiate by DMSO, the Ki-67 negative fraction, as well as the G0/G1 DNA fraction, was increased in comparison to an undifferentiated control.

Methods of staining nuclear suspensions for bivariate Ki-67 antigen/DNA and bromodeoxyuridine/DNA analysis wer elaborated to minimize cell losses by the avoidance of fixation and washing, and could be performed only on 10^4 to 10^5 frozen cells/sample.

VACCINIA/POLYOMA RECOMBINANT VIRUS : A MODEL FOR TUMOUR IMMUNITY

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Many tumour cells present novel antigens. Tumour-specific antigens (TSA) have been exploited in the diagnosis and imaging of human carcinoma and the administration of anti-TSA antibodies has met some measure of success in the treatment of clinical disease. We have investigated, in a model system, the possibility that expression of TSA from a live recombinant virus might stimulate the host itself to mount an anti-tumour immune response.

Cells transformed by polyoma virus (PY) express three protein species from the integrated viral genome: LT, MT and ST. However, the exact relationship between the early PY protein species remains unclear. We thus constructed vaccinia virus recombinants separately expressing the three PY proteins.

Cell lines infected with the live recombinant viruses express high levels of the T proteins although cell surface fluorescence using anti-T serum was not detected. In all cases the recombinant T proteins exhibit biochemical activities associated with the authentic PY proteins.

Rats injected with syngeneic PY-transformed rat cells rapidly develop discrete tumours. Animals inoculated with the vaccinia recombinant expressing ST failed to reject transplanted tumour cells whereas animals previously vaccinated with recombinants expressing either MT or LT subsequently rejected their tumours. Further, animals already bearing tumours could be induced to reject their tumour cells by vaccination with the appropriate recombinant.

EFFECT OF GROWTH FACTORS ON FILM SARCOMA

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Film sarcoma is provoked by unabsorbable films, e.g. nitrocellulose filters, implanted in rodents. Substances adsorbed to the film can influence tumour growth. The cell-type of origin of the tumour has not been unequivocally determined. Growth fctors obtained from different types of cell were tested on this system to see if the response varied with the origin of the growth factor. Five groups of 50 female BALB/c mice were implanted subcutaneously with 25 mm filters bearing fibroblast (0.1 microgram), epidermal (0.2 microgram), interleukin 2 (2 units), nerve growth factor (0.002 mg) and saline respectively, and observed weekly for tumour growth. The yield of tumour in each was comparable to the controls, with the exception of nerve and interleukin factors, where yield varied by 25%. Differences were not statistically significant.

Growth factor	Mice	Tumours	Total weeks of life	Mean weeks of life/ tumour
Interleukin 2	47	25	2194	88
Fibroblast	49	26	2554	98
Control	50	23	2615	114
Epidermal	50	21	2559	122
Nerve	46	16	2149	134

PURIFIED TUMOUR ANTIGENS FROM MURINE SARCOMAS

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Two functionally similar TSTAs (Tumour Rejection Antigens) have been purified to chemical homogeneity from several chemically-induced murine sarcomas. p82, a unique antigen not previously described and p86 antigen, showing homology with heat shock proteins, are distinct entities but each is highly immunogenic and specific for the tumour of origin. Methods used for extraction and purification, biochemical properties, cloning of the gene encoding for p86, and immunogenic characteristics of these tumour antigens have been investigated and defined.

GROWTH FACTOR REQUIREMENTS OF NORMAL HUMAN MESOTHELIAL CELLS

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