for 30 days. In these conditions, PBL proliferated but did not develop cytotoxic activity against Auto-Me and K562 cells. The phenotype of PBL at the 30th day was 95% CD3, 95% CD4, 1% CD8 and 0% CD16. Clones were then derived at 1 cell/well in the presence of irradiated Auto-Me, RIL-2 (25 U/ml) and Daudi cells as feeder. The 81 growing clones were screened for cytotoxicity and proliferating activity in the presence of Auto-Me. Twelve clones were cytotoxic for Auto-Me and 22 clones showed significant proliferation with Auto-Me; 67 clones exhibited both cytotoxic and proliferating activity. Preliminary results of the specificity analysis showed that one clone which proliferated to Auto-Me expressed cytotoxicity on Auto-Me but not on 12 different targets including autologous EBV-B cells and fibroblasts, 6 allogeneic melanomas, 2 lymphoblastoid lines, Daudi and K562.

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BIOCHEMICAL EVALUATION OF HYPERCALCAEMIA ASSOCIATED WITH BRONCHOGENIC CANCER

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Bronchogenic carcinoma (BC) may be frequently associated with hypercalcaemia, due to direct bone resorption by tumour cells, or alternatively by osteoclastic bone resorption stimulated by humoral factors (i.e. parathyroid hormone (PTH)-like substances and prostaglandins (PGE)).

In 160 patients with BC, hypercalcaemia was found in 19 cases (11.8%). By measuring the biological parameters of PTH activity and the circulating levels of PTH and PGE the hypercalcaemic patients were divided into three groups. The first group (n=6) presented the typical biochemical pattern of hyperparathyroidism, the second group (n=10) was characterized by high circulating levels of PGE; in the third group (n=3) all the parameters considered were normal. A metastatic bone involvement as evidenced by radiologic and scintigraphic means, was documented only in patients of the first and second group. These data further emphasize the importance of humoral factors in the pathogenesis of hypercalcaemia associated with BC.

NUCLEIC ACID BINDING OF TRANS-4-AMINOSTILBENE DERIVATIVES *IN VITRO* AND *IN VIVO*

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Trans-4-acetylaminostilbene (AAS) is a strong tumour initiator in rat liver. The model ultimate carcinogen, N-acetoxy-AAS, reacts with nucleic acids *in vitro* and predominantly with guanine. The major adducts are four cyclic isomers in which guanine is substituted at N2 and N3 as shown by reactions with Guo and d-Guo. In addition, two minor guanine adduct fractions were identified and shown to consist also of sets of isomers. After oral administration of trans-4-dimethylaminostilbene (DAS) and AAS, the cyclic adducts and presumably also the minor adducts are formed in rat liver DNA. Substitution of guanine at N2 and N3 labilizes the glycosidic bond, which results in depurinization of DNA, and may impair nuclease activity, which could explain the observed incomplete hydrolysis of modified DNA. Experiments with AAS labelled in the acetyl group indicate that non-acetylated adducts are also generated in liver RNA and DNA to some extent. Among these non-acetylated adducts the cyclic guanine adducts are also present. A number of persistent adducts could be demonstrated 28 days after oral administration of DAS.

CORRELATION OF ELASTOSIS WITH SOME MORPHOPATHOLOGICAL PROGNOSTIC FACTORS IN BREAST CARCINOMAS

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The increase of elastic tissue was found on haematoxylin-eosin stained tumour sections in 54 (22%) from 245 invasive ductal carcinomas of the breast treated by radical mastectomy, as a pink, homogeneous or finely fibrillar sheaths around carcinomatous ducts and focal deposits in contact with tumour parenchyma. The elastosis was assessed subjectively in three degrees and analysed in relation to the tumour size, histological pattern, grade of malignancy, lymph node metastases and age of patients. The percentage of elastin positive tumours increased in parallel with their histological differentiation from trabecular carcinomas to pure glandular carcinomas. Elastosis correlated to some extent with the grade of malignancy - a higher proportion of elastin positive tumours was found in low grade group and conversely, a higher proportion of cases without elastosis in high grade tumours. No relation was established between elastosis

and lymph node status. The percentage of positive tumours slightly increased with patients' age. It seems that elastosis has a limited value as a single prognostic factor.

ANTIGENS EXPRESSED IN VIVO FROM THE BamH1 W FRAGMENT OF EPSTEIN BARR VIRUS

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A gene bank was prepared in a plasmid expression vector from small fragments of Epstein Barr Virus (EBV) DNA. When screened with serum from patients known to be immunopositive for EBV, a number of colonies which expressed EBV antigens were identified. Subsequent screening of these colonies with a probe for the repeated BamH1 W fragment showed that 4 of them originated from that region of the EBV genome.

The exact locations of the antigen coding sequences were established by DNA sequencing. All correspond to parts of potential open reading frames which had previously been identified by sequence analysis. These are in three different reading frames and two of the sequences, which are in different reading frames, contain overlapping sequences. The DNA fragments identified in this study do not correspond to exons shown previously to be part of EBNA2 or EBNA5.

DEREGULATION OF THE TYROSINE KINASE ASSOCIATED WITH THE BOMBESIN RECEPTOR IN SMALL CELL LUNG CARCINOMAS

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It has been hypothesized that bombesin-like peptides produced by small cell lung carcinomas (SCLC) may sustain deregulated proliferation through an autocrine mechanism. We have recently identified, using phosphotyrosine antibodies, a 115 kD tyrosine kinase (p115) which is part of the bombesin receptor complex in mouse Swiss 3T3 fibroblasts (Cirillo D., Gaudino G., Naldini L. and Comoglio P.M., Mol. Cell. Biol. 6: 4641-4649). We now report that phosphotyrosine antibodies recognize a protein of 115 kD, phosphorylated at tyrosine, in four human SCLC lines producing bombesin, but not in a non-producer "variant" line. P115 from detergent treated SCLC does bind to bombesin-Sepharose and becomes phosphorylated at tyrosine in the presence of radiolabeled ATP and MN++ ions. As in the case of p115 kinase immunoprecipitated from mouse fibroblast, the SCLC p115 is phosphorylated in an immunocomplex kinase assay. However, the latter does not require the presence of exogenous bombesin activity. Binding data, obtained using radiolabelled ligand, indicate receptor occupancy in the cell lines producing bombesin. These observations fulfill the hypothesis of autocrine control of human small cell lung carcinoma cell proliferation, via constitutive activation of bombesin receptors.

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GROWTH FACTOR PRODUCTION BY NORMAL HUMAN MESOTHELIAL CELLS AND MESOTHELIOMA CELL LINES

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It has been shown that normal human mesothelial cells (NHM) blocked in G1 by serum starvation can be induced to undergo a round of DNA synthesis either by TGF-beta or by PDGF. Seven human mesothelioma cell lines were compared to NHM primary cultures by northern analysis for their level of expression of mRNA hybridizing with cDNA for PDGF A chain, PDGF B chain (c-sis) or TGF-beta. NHM cells produced no detectable B-chain mRNA and low levels of A chain message. Mesothelioma cell lines produced high levels of either A or B chain message or both. Both normal and tumour cells produced TGF-beta mRNA. NHM cells transformed by transfection with an SV40 T antigen construct produced levels of message for all three growth factor genes which fell within the normal range of hybridization analysis. Mesothelioma lines but not NHM cells are positive for PDGF by immunoprecipitation or bioassay of conditioned medium. Thus, mesothelioma cell lines produce a growth factor which is mitogenic for NHM cells. An autocrine role of PDGF in tumourigenesis is being investigated.

ROLE OF HOST RESPONSES IN THE DRUG TREATMENT OF METASTASES

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