SUPPRESSION OF NATURAL ANTI-TUMOR MECHANISMS BY TUMOR-PROMOTING AGENTS

R. Keller, Immunobiology Research Group, Institute of Immunology and Virology, University of Zurich, Switzerland

The process of chemical carcinogenesis is considered to proceed in various tissues in at least 2 sequential stages : initiation and/or transformation by solitary carcinogens, producing irreversible tissue alterations, and enhancement of outgrowth of transformed cells by tumor-promoting agents. However, the mechanisms by which solitary carcinogens and tumor promoters lead to oncogenesis are insufficiently defined. In showing that tumor-promoting polyfunctional diterpene derivatives of the tigliane, ingenane and daphnane type prevent the enhancement of natural cytotoxicity in resting macrophages (activation step) and suppress the manifestation of natural cytotoxicity by previously activated macrophages (effector step) and by "Natural Killer" cells, and moreover enhance tumor growth in vivo, the present findings lend support to the concept that tumor promoters function primarily via interference with natural antitumor effector systems. Solitary carcinogens exerted little or no activity in these experimental systems.

ULTRASTRUCTURE OF HUMAN NATURAL KILLER CELLS

R.E. Merchant and St. Arrenbrecht, Dept. Innere Medizin, Abt. Onkologie, Universitätspital Zürich, 8091 Zürich

In an effort to better define the morphological characteristics of natural killer (NK) cells, freshly isolated human peripheral blood mononuclear cells (MNC) were depleted of T lymphocytes as well as of phagocytic or plastic-adherent cells. The resultant fractions showed high levels of cytotoxicity to a human hypernephroma cell line (as high as 98 % specific 51-Chromium release at a 4:1 MNC-to-target cell ratio) and, therefore, represented populations enriched for NK activity. In these cultures, MNC adhering to living or glutaraldehyde-fixed tumor cells were assumed to be predominately NK cells and were examined by scanning and transmission electron microscopy. Adherent cells were round (approx. 4 μm dia.) and possessed variable numbers of microvilli. Internally, these cells possessed a large, heterochromatic nucleus (nucleus:cytoplasm ration $>$ 1) and a cytoplasm containing numerous mono- and polyribosomes, a few large mitochondria, an occasional electron-dense granule, a well developed Golgi apparatus and a variable number of clear vesicles near the cell surface. Microvilli of these cells contained only cytosol and were often branched. At areas of NK-tumor cell contact, the target cell's surface was indented by pseudopodia of the NK cell. Although intimate contact was achieved, an intercellular space of irregular width was always maintained and no focal plasmalemmal alterations such as specialized junctions, membrane fusions or deletions were observed. Furthermore, the cytoplasm within the NK cells' pseudopodia did not differ significantly from the cytoplasm in the remainder of the cell. Intercellular contacts were similar irrespective of whether the tumor cell was viable or glutaraldehyde-fixed. Thus while we can now define the essential morphology of cells which in all probability are natural killer cells, the structural basis of NK-mediated cytotoxicity remains unclear. The mechanism appears to involve no alteration of the component plasmalemmas of either cell or the release of lysosomal enzymes by NK cells at the zones of contact.

IN VITRO SENSITIVITY OF HUMAN TUMOR CELLS TO SPONTANEOUS CELL MEDIATED CYTOTOXICITY (SCMC)

M. Fopp, M.E. Weber, H.J. Senn, Department of Medicine, Division of Hematology and Oncology, Kantonsspital St-Gallen, Switzerland

The correlation between in vitro sensitivity of tumor cell lines to spontaneous cell mediated cytotoxicity (SCMC) or natural killer activity (NK) and in vivo tumor formation in transplantation experiments in mice indicates that SCMC might be involved in the early stages of tumor development. Whereas the effector mechanisms of SCMC were extensively analysed, few data exist on susceptibility of uncultured human tumor target cells from different origin.
In a short term 51-Cr release assay we tested freshly seperated, shortly incubated as well as frozen tumor cells from the same patients with hematologic neoplasia and solid tumors for their susceptibility to NK-lysis from healthy unprimed donors with a high lytic capacity. Solid tumors were separated by enzyme treatment avoiding

trypsinisation. The K562 cell line from a patient with chronic myeloid leukemia in blastic crises was used as a positive control target due to its particular susceptibility to SCMC. Results are shown comparing curves of a series of effector to tumor target cell ratios with the corresponding positive control target tested at the same time.
51-Cr uptake of tumor cell suspensions from patients with acute leukemia and lymphoma was significantly lower compared to solid tumors. There was no difference with respect to spontaneous 51-Cr release during the test. Four out of 26 freshly separated tumor cell suspensions showed some lysis. The highest values were observed in tumor cells from a cerebellar metastasis of a lung tumor patient. Data were reproducible and there was no difference between fresh and frozen tumor cells neither in positive nor in negative test results. The other three tumor suspensions with very low NK-sensivity were from neoplastic ascites of patients with ovarian, stomach and breast cancer. Possible explanations for this low sensivity of uncultured human tumor cells will be presented.

KINETICS OF INTERFERON INDUCED INHIBITION OF DNA SYNTHESIS

Z. Schneider and St. Arrenbrecht. Dept. Innere Medizin, Abt. Onkologie, Universitätspital Zürich, Switzerland

22 hour preincubation of human mammary carcinoma cells (BT 20) with interferon (IFN) inhibited in a dose dependent fashion, the incorporation of 3-H-thymidine (3-H-T) into acid insoluble material (DNA synthesis). Maximal inhibition was seen with 1000 U IFN/ml, and was comparable to that observed after treatment with Actinomycin D (10 µg/ml). Uptake of 3-H-T into an acid soluble pool was only marginally affected. The kinetics of the induction of DNA synthesis inhibition were followed by hourly pulses of 3-H-T over a period of 22 hours, starting at the time of IFN addition to the cultures. We observed an initial lag period followed by a short-term increase in DNA synthesis. This burst of DNA synthesis occurred earlier when higher doses of IFN were used. An IFN dose dependent inhibition of DNA synthesis was seen only 12 hours after IFN treatment. Control values were again observed after 44 hours. We saw no parallelism between uptake of 3-H-T and DNA synthesis. Another human cell line (hypernephroma) showed also a lag period followed by a burst of DNA synthesis after IFN treatment. However, only a marginal inhibition was seen later. Thus, IFN enhances and then - in susceptible cells - depresses DNA synthesis. The inhibition by IFN is reversible. These effects are not merely a consequence of fluctuations in 3-H-T transport through the cell membrane.

STEROID RECEPTORS IN HUMAN MAMMARY TUMORS; RESULTS AND EXPERIENCE OF FOUR YEARS

U. Eppenberger, A. Takahashi, A. Alemdral, J. Torhorst and W. Roos, Hormone Laboratory, Dept. of Gynecology, Kantonsspital Basel, Switzerland

During the last four years estrogen and progesterone receptors of more than 800 mammary tissue biopsies were measured in our laboratory. The method used is a five-point saturation assay based on the Dextran charcoal procedure using 3H-estradiol competed by diethylstilbestrol for the estradiol receptor and 3H-R-5020 competed by progesterone for the progesterone receptor. The evaluation is carried out by computer-aided Scatchard plot analysis. Receptor concentrations of more than 10 fmol/mg protein are regarded as positive.
From 480 mammary carcinoma biopsies 49 % were positive with respect to both receptors (ER+/PgR+), 23 % contained only estrogen receptor (ER+/PgR-, 23 % were negative for both receptors (ER-/PgR-), whereas in 5 % only progesterone receptor was beyond the detection limit (ER-/PgR+). In metastases of lymphnodes (n-21) the receptor-containing tissues were less frequent (ER+/PgR+: 19 %; ER+/PgR-: 38 %; ER-/PgR+: 0 %; ER-/PgR-: 43 %). The percentage of ER+ samples increased with age. Premonopausal women tend to have lower ER-concentrations, showing an increase in percentage which lacks both receptors.
The same method is used by five laboratories in Switzerland being members of the steroid receptor study group of the SAKK (Schweizerische Arbeitsgruppe für klinische Krebsforschung). Quality controls are regularly carried out by sending out standard preparations which are assayed by all participants of the SAKK study group. The results are consistent with respect to the quality judgment, whereas quantitative agreement did not prove satisfactory.
The tissue selection by the pathologist is of utmost importance. All tissue samples tested for estrogen receptors were submitted to an additional histological examination. In 7 % of all cases the result of this examination was different from the original histological diagnosis, accounting for a substantial part of receptor-negative cases.
Estrogen receptor determination on dysplastic tissue resulted in a correlation between the presence of estrogen receptors and morphological high risk factors.
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