Abstracts of Theses from the Scandinavian Countries

Abstracts of Scandinavian theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Light microscopy and morphometry of vinblastine in vivo cytotoxicity in the different developmental stages of rat incisor ameloblast epithelium

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Vinblastine (VB) is assumed to preferably kill cancer cells and normal cells in mitosis. It is also assumed that this cell killing is obtained by dissolvement of the threads in the mitotic spindle by depolymerization of the microtubular protein or by inhibition of the formation of the microtubular protein or its precursors. Some investigations in vivo have, however, shown that a large dose of VB can kill also normal, mature, non-dividing cells and, moreover, inhibit their function. In the present investigation VB-induced cell death and functional inhibition were studied in a normal cell population with cells in different phases of differentiation. The enamel-producing epithelium of the rat front tooth was used as a suitable model since the different phases of cellular differentiation are located in a clear linear sequence and easily can be identified morphologically. The secretory function can also be morphologically evaluated as the secretion product (enamel matrix) is deposited in loco. By a special method topographically controlled tissue sections were taken from the ameloblast epithelium. The effects of VB were registered by conventional observations on light microscope level. With a video light microscope the degree of selected VB-effects was registered by quantitative stereological methods (morphometry). After i.v. injection of VB, dose-effect curves for cell death and for the ability of functional recovery were registered within the different cell phases as stem cells, proliferating ameloblasts, differentiating and mature ameloblasts. By this method the relative sensitivity of the different cell phases could be evaluated. Supplementary studies were performed on dividing cells in intestinals crypts and mature exocrine secretory cells in pancreas of rats.

The investigation confirmed that dividing cells are the most sensitive to VB. It showed, however, also that VB, at lower doses than previously assumed, to a considerable extent killed or inhibited the function of non-dividing, maturing or mature cells in the ameloblast epithelium. The tendency to cell death was, however, less pronounced in the exocrine, secretory pancreatic cells but the secretory function was temporarily inhibited even in these cells. The author proposes the hypothesis that the sensitivity of different cell phases to VP is a function of depolymerization of the cytoplasmatic microtubuli and inhibition of the ribosomal protein synthesis.

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Prognostic factors in urinary bladder cancer with special reference to a modified grading system, flow cytometry, and tissue polypeptide antigen

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The natural course of human cancer is highly variable. The therapeutic strategy is mainly guided by tumor grade and stage.

For almost two decades urothelial cancers have been classified according to the WHO system. WHO grade I tumors usually have a favorable course with a nearly 100% 5-year survival, whereas the WHO grade III cancers are highly malignant with early invasive growth and metastatic potential. The WHO grade II cancers present great difficulties for the choice of therapy warranting efforts to better classify this heterogeneous subgroup of tumors.

In a retrospective study of 124 newly diagnosed bladder cancer patients who were followed for at least 5 years a reclassification was carried out. A subdivision of the WHO grade II cancers was performed into grades IIa and IIb with the ambition to concentrate the criteria on objective parameters possible to assess in numerical terms namely variation in nuclear size and frequency of mitoses. The 5-year survivals were 92 and 43% respectively, in grades IIa and IIb. A new classification was proposed in which grades I and IIa were embraced into grade A, grades IIb and III into grade B, and the anaplastic cancers called grade C. The 5-year survival was 94, 44 and 27% in grades A, B and C respectively.

The reproducibility of the proposed system was comparatively high with an inter- and intrapersonal variation in grading of less than 10%.

In a multivariate analysis, comparing tumor stage, histological and cytological grade, DNA content and the proportion of cells in a proliferative phase (S-phase), the proposed new grading system seemed best related to prognosis. No other variables contributed significantly to predict the final outcome for the patient.

Elevated levels of tissue polypeptide antigen (TPA) have been reported to be related to human bladder tumor activity. The presence of TPA in human urothelium was analyzed with immunohistochemical techniques in normal, inflamed and cancerous tissues. The concentration of TPA was found to be similarly distributed in the different types of urothelium. It was concluded that the elevated TPA levels found in serum or urine were probably due to an increased cell turnover and autolysis.

In a prospective study the presence of TPA in urine (U-TPA) was analyzed with a commercially available radioimmuno assay (RIA) technique in 81 patients with a known diagnosis of urothelial carcinoma. A good correlation was noted between U-TPA levels, cytological grades, DNA-content and tumor stage. Of the three parameters a multivariate analysis indicated U-TPA to be best related to tumor invasiveness. Measurement of urinary TPA may, hence, become a simple and objective means to signal the occurrence of aggressive tumor populations in urothelial carcinoma.

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Molecular studies of human chromosome 22 with particular reference to its involvement in the oncogenesis of meningioma

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Meningiomas, tumors of the membranes covering the central nervous system, show several elementary features similar to retinoblastoma. Retinoblastoma—a tumor of the retina which occurs in children—is the fundamental example of the recessive mechanism in oncogenesis and it involves two mutations of a gene on the long arm of chromosome 13. It appears likely that a similar recessive mechanism operates in meningioma. Several lines of

evidence support the idea that there is a gene on chromosome 22, which is involved.

In an attempt to localize a tentative meningioma gene on chromosome 22, by deletion mapping of tumor DNA, a series of 81 sporadic meningiomas were studied using restriction fragment length polymorphism (RFLP) analysis. Two types of aberrations affecting chromosome 22 were detected in the tumor DNA; monosomy 22 and variable terminal deletions of the long arm of one chromosome 22. By using the information derived from tumors with terminal deletions of 22q, a tentative meningioma gene has been localized to the region distal to the myoglobin (MB) locus, which corresponds to 22q12.3-qter. Bilateral acoustic neurofibromatosis (NF-2) is a heritable disease which includes an increased risk of the development of meningioma. The putative gene responsible for NF-2 has also been localized to chromosome 22 and it has been proposed that a common pathogenetic mechanism involving a locus on this chromosome, might be involved in both conditions. However, recent results suggest that the NF-2 gene is located proximal to the MB locus. Thus, it would appear that at least two different loci are involved in the origin of these disorders.

In order to facilitate the more precise localization of the meningioma gene, 195 chromosome 22-specific DNA probes, corresponding to more than 1% of this chromosome, were isolated employing two different approaches. Mapping of these probes to chromosome 22, and subsequently to one of its four different regions, was made possible by hybridization to a panel of five somatic cell hybrids. Twenty-eight of the probes, which were localized to the region 22q12-qter, detect RFLPs. These new markers will make a significant contribution to the construction of a tight genetic linkage map as well as a long-range physical map of the long arm of chromosome 22. In order to identify smaller rearrangements on the long arm of this chromosome, the new chromosome 22-specific probes will be important tools in the further analyses of meningiomas.

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Lymphocyte kinetics and function in Hodgkin's disease—A clinical and immunological study

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The prognostic discriminatory capacity of blood T cell abnormalities in adult Hodgkin's disease (HD) was studied. Pretreatment lymphocytopenia and T cell functional abnormalities revealed as an increased spontaneous and a decreased concanavalin A induced blood lymphocyte DNA synthesis predicted a poor prognosis. In multivariate analyses abnormal lymphocyte function was the strongest prognostic factor besides age.

The serum concentration of the soluble form of the CD8 antigen (sCD8), a surface membrane component of suppressor/cytotoxic T cells was measured in patients with untreated HD. The mean sCD8 levels were higher in patients with clinical characteristics associated with a poor prognosis. Furthermore, patients with abnormal lymphocyte function had raised sCD8 values. Actuarial survival of patients with high sCD8 levels was worse than that of patients with normal or moderately increased sCD8 values.

Pneumococcal septicemia is a life-threatening complication in splenectomized individuals. This infection is more frequent in splenectomized HD patients than in patients splenectomized for other reasons. However, in HD the antipneumococcal antibody levels were normal before vaccination both in untreated patients and in patients in long-term remission irrespective of a previous splenectomy. In splenectomized HD patients the antibody response to pneumococcal vaccination did not differ from that of splenectomized patients with other diagnoses. Moreover, antibody levels after vaccination did not differ between patients with HD and subjects splenectomized after traumatic rupture of the spleen as analysed up to 3 years post vaccination. HD patients vaccinated before splenectomy and institution of therapy had a better antibody response than remaining HD patients.

One mechanism contributing to the impaired cell mediated immunity and reduced number of circulating T cells (mainly $CD4^+$ cells) in HD may be a sequestration of T cells in tumour involved tissue. To test this hypothesis patients with active disease and patients in clinical complete remission were studied. Autologous blood lymphocytes were labelled with indium-111 oxine and infused intravenously. The radionuclide bound to $CD4^+$ and $CD8^+$ blood lymphocytes was measured in serial blood samples. $CD4^+$ cells were eliminated more rapidly from the blood in patients with active disease than in patients with no demonstrable tumour. The enhanced clearance of $CD4^+$ cells in patients with active HD may contribute to the observed blood T lymphocytopenia and the functional T cell deficit. Repeated gamma camera imaging after infusion of labelled cells demonstrated an accumulation of radionuclide in tumour involved tissue in patients with active disease.

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Genomic alterations in renal cell carcinoma and prostate carcinoma with special reference to inactivation of tumor suppressor genes

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Renal cell carcinoma (RCC) and prostate carcinoma (PC) are common urological tumors in man. Their etiology is unknown. Current treatment of these tumors is far from satisfactory. Research to elucidate the mechanisms underlying the malignant phenotype of these tumor cells is needed to form a scientific basis for new effective therapeutic strategies.

Malignant disease is caused by genetic damage to cells. The details of these changes have until recently been quite obscure. Progress in molecular genetic research has led to the isolation of proto-oncogenes, oncogenes and tumor suppressor genes (TSG). Proto-oncogenes are activated to oncogenes by dominant genetic mechanisms while loss of function of tumor suppressor genes by recessive mechanisms contribute to the malignant phenotype. With this background we used molecular genetic techniques as well as karyotype analyses to investigate the occurrence of genetic changes in tumor tissue from sporadic cases of human RCC and PC. Kidney tumors from two patients with von Hippel Lindau disease (who develop RCC as an inherited trait) were also included.

Specific chromosomal deletions were detected in these tumors. In RCC the short arm of chromosome 3 (3p) was lost in 68% of cases. From a common deleted region on 3p, 3p21, which suggests the localisation of a tumor suppressor gene, we isolated an anonymous gene that is expressed in normal renal tissue but at drastically reduced levels in tumor tissue, in addition variable frequencies of deletions on other chromosomes were also detected in RCC most commonly involving chromosomes 5 and 14. In PC the short arm of chromosome 8 and the long arm of chromosome 16 showed the highest frequencies of deletions (63% and 56% respectively). The average number of deletions on all chromosomes was highest in the most aggressive tumors.

A relatively high frequency of deletions on chromosomes 10q and 18q was found in both RCC and PC which suggests a more general involvement of deletions in these regions.

These results are in accord with the thesis that a major mechanism in the oncogenesis of these tumors involves somatic mutations which inactivate tumor suppressor gene functions. The tentative TSGs involved differ for the two tumor forms but there may be some common components. These results are similar to those from studies of other tumor forms where such findings have led to the isolation of tumor suppressor genes.

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Intracellular pharmacokinetics and in vitro effects of anthracyclines on human leukemic cells—Relation to treatment results in acute non-lymphoblastic leukemia

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Aim: To find methods to optimize and individualize the chemotherapy of acute non-lymphoblastic leukemia by studies in vivo and in vitro of the interactions between drugs and leukemic cells from patients.

Methods and Results: The pharmacokinetics of three anthracyclines, daunorubicin, doxorubicin and an epimer of doxorubicin, epirubicin, were studied in leukemic cells. Drug concentrations were determined by HPLC. Daunorubicin reached the highest intracellular peak concentrations, followed by epirubicin and doxorubicin. In contrast, doxorubicin had the longest and daunorubicin the shortest intracellular retention. These results can explain the differences in clinical activity of the three drugs. Epirubicin was significantly less toxic to normal bone marrow cells than doxorubicin with a retained effect to leukemic cells. This indicates a better therapeutic index for epirubicin.

Also conjugation of doxorubicin to a macromolecule, DNA, induced pronounced changes in the pharmacokinetics of this drug. The concentrations in leukemic cells in vivo were increased by 60% and the V_d was reduced from about 25 L/kg to 7 L/kg. The clinical relevance of this was evaluated in a randomized multicenter trial of doxorubicin versus doxorubicin-DNA. Patients treated with the doxorubicin-DNA conjugate had a significantly improved survival (27.3 versus 12.1 months; $p \leqslant 0.001$) and duration of first remission (23.6 versus 13.2 months; $p \leqslant 0.025$) and also significantly fewer cardiac and intestinal side effects.

The results from the studies of intracellular pharmacokinetics were used to establish how leukemic cells should be incubated in vitro to mimic what is obtained in vivo during therapy. These incubation conditions were used to evaluate a vital dye exclusion assay, the DiSC assay, as a tool for in vitro studies of antileukemic drugs and as a predictive test for response to chemotherapy. There was a significant correlation between the in vitro effect of anthracyclines and ara-C, and the patients response to therapy ($p \leqslant 0.005$ and $p \leqslant 0.05$ respectively) and the assay showed a predictive accuracy of 90% for both drug sensitivity and resistance.

In the last part of the study, this modified DiSC assay was used to address the problems of how tumor cells develop resistance to cytostatic drugs. The possibility of increased active transport of anthracyclines out from the cells was studied by incubation of fresh leukemic cells with anthracyclines, alone and in combination with a calcium channel blocker, verapamil. Both the intracellular uptake and cytotoxic effect of anthracyclines in vitro were significantly increased by verapamil. Another possible mechanism for drug resistance, increased drug metabolism, was evaluated by immunocytochemical studies of the expression of glutathione transferase π in leukemic cells taken from patients at different stages of the disease. The expression of this enzyme was significantly correlated to the subsequent treatment results, survival and duration of first remission.

Conclusions: 1. Studies of intracellular pharmacokinetics can help to improve the therapy for patients with acute non-lymphoblastic leukemia. 2. The DiSC assay, used with in vivo mimicking incubation conditions, is a relevant tool for in vitro studies of antileukemic drugs and may be used to individualize therapy. 3. Both increased drug metabolism and drug efflux, which can be counteracted in vitro, are clinically relevant mechanisms for drug resistance.

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Clinical and experimental studies on uptake of radiolabeled 2-fluoro-2-deoxy-D-glucose during cancer treatment

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Uptake of 2-fluoro-2-deoxy-D-glucose (FDG) in cancer patients, neoplastic cells and tumor-bearing mice was studied in order to elucidate the role of FDG in assessing the response of tumors to cancer treatment. The ultimate goal is to develop a non-invasive predictive test that could be used as an adjunct to conventional and novel determinants of radiosensitivity. Another goal is to use FDG imaging as a diagnostic and prognostic tool with positron emission tomography (PET).

In all, 34 patients with head and neck tumor and 17 patients with advanced breast cancer were studied with a special gamma camera collimated for detection of high energy (511 keV) photons. FDG, labeled with the positron emitting radionuclide fluorine-18, was avidly taken up by both head and neck tumors and breast cancer metastases. In 13 head and neck tumors FDG uptake correlated strongly with proliferative activity, as assessed by DNA flow cytometry.

Nineteen patients with head and neck tumors and 10 patients with breast cancer were scintigraphied twice to study treatment effects. Radiotherapy caused a decrease of FDG uptake in all but two head and neck tumors that were both refractory to treatment. The decreased uptake at 30 Gy seemed to reflect the antiglycolytic effect of irradiation per se rather than a good clinical outcome. Generally chemotherapy, which was administered only to the breast cancer patients, resulted in similar, though less clear-cut changes. [^{18}F]FDG scintigraphy was the first method to reveal in-field recurrence of irradiated axillary breast cancer metastasis.

Cell cultures and intramuscularly grown mouse Lewis lung tumor (LLC) were used to study drug effects. FDG uptake was related to the intracellular ATP content in vitro in two human ovarian cancer cell lines and murine L1210 cells treated with methotrexate (MTX), cisplatin and toremifene. Except for MTX, FDG uptake exhibited clear dose-response curves with increasing drug concentration. The relationship between FDG uptake and ATP production suggested that these cell lines derive most of their energy from glycolysis, although other pathways may be involved to a lesser extent.

BCNU caused regression of LLC, while doxorubicin (ADM) was ineffective in this tumor model. The association of [^{14}C]FDG uptake between tumor response and changes in DNA ploidy was highly significant. By high performance liquid chromatography (HPLC) 45 min after injection, most of the FDG in the tumors had been phosphorylated (>75%), which is consistent with the metabolic accumulation of FDG as FDG 6-phosphate. Glucose

6-phosphatase enzyme histochemistry (G6-Pase EHC) showed that BCNU treated tumors retained minor G6-Pase activity, indicating dephosphorylation of FDG in these tumors.

This study is in line with earlier experimental and clinical observations which indicate that FDG is a good general purpose tumor detecting radiopharmaceutical. Follow-up of tumor response during cancer therapy can be accomplished by serial imaging with FDG. However, the real value of the procedure should be weighed against the costs and the sophisticated technology needed, i.e. the clinical used of PET. To validate PET-FDG method as a prognostic or predictive tool in oncology, a quantitative analysis of FDG uptake is mandatory.

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Clinical, histological and cytometrical findings in oral cavity carcinomas and their therapeutic and prognostic significance

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Nearly 60% of patients with oral cavity carcinomas presented with advanced stage of disease. The duration of symptoms was shorter in patients with large tumours than with small ($p < 0.001$), and in the patients with metastases than in those without ($p < 0.01$). The clinical assessment of lymph node status yielded a fairly high percentage of false negative results. CT and MR should be more regularly performed to assess the neck.

Malignancy grading score (Jakobsson et al., 1973 and Glanz & Eichhorn, 1985) had a positive correlation with the initial lymph node status at presentation ($p < 0.05$), but not with tumour T stage. Histological differentiation did not correlate with either tumour T-, or N-status.

Fifty-five per cent of oral cavity carcinomas showed a non-diploid DNA content. Heterogeneity in DNA content does not seem to be a major problem. DNA ploidy correlated with tumour size ($p < 0.01$), frequency of bone invasion ($p < 0.001$), lymph node status ($p < 0.001$) and histological differentiation ($p < 0.001$). DNA aneuploid tumours responded better to preoperative radiotherapy than did DNA diploid ($p < 0.01$) and DNA polyploid tumours ($p < 0.05$).

Tumour size ($p < 0.01$), lymph node status ($p < 0.001$) and tumour DNA ploidy ($p < 0.005$) were the only independent prognostic factors.

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Familial hemophagocytic lymphohistiocytosis—A clinical, metabolic and immunological study of a lymphohistiocytic inflammatory disorder

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Familial hemophagocytic lymphohistiocytosis (FHL) is a potentially fatal disease characterized by fever, hepatosplenomegaly, cerebral symptoms, cytopenia, coagulopathy, and hypertriglyceridemia. The incidence of FHL in Sweden during the 16-year period 1971–1986 was estimated in a retrospective study at 1.2/1 000 000 children per year; which corresponds to one child per 50 000. Clinical, laboratory, and pathological features in 32 children with FHL were reviewed. The disease was underdiagnosed, and fewer than half of the children (11/32) were diagnosed while alive.

In a clinical study, a therapy regimen, which was based on teniposide, etoposide, steroids, and intrathecal methotrexate, was developed. More than half of the children are still alive, three of them with more than 4 years' survival after diagnosis. For diagnostic purposes, fine-needle aspiration biopsy of the spleen was found to be a valuable method to reveal the hemophagocytic activity, both at onset and during relapses.

During active FHL, triglycerides were markedly elevated in serum, in very low density lipoproteins, and in low density lipoproteins, whereas both triglycerides and cholesterol were reduced in high density lipoproteins. The lipoprotein abnormalities, reversible with successful therapy, were compatible with a depressed lipolytic activity. Postheparin levels of lipoprotein lipase (LPL) and hepatic lipase were found to be very low during active FHL. The results suggest that inflammatory cytokines, such as tumor necrosis factor-alpha (TNF) which strongly suppress LPL activity, may be of major pathophysiological importance in FHL.

A method to detect single TNF-producing cells, based on immunofluorescence technique and specific monoclonal antibodies, was developed. A well-defined staining of intracellular TNF with a local, polar accumulation in a juxtanuclear position of the cell, most probably the Golgi complex, was found. At the peak of the response, which occurred 2–3 h after lipopolysaccharide exposure, 50–60% of the blood monocytes produced TNF.

Serological studies revealed an increase in TNF and interleukin-6 during active FHL as well as a marked elevation in interferon-gamma and soluble CD8. It is suggested that FHL is caused by a genetic defect in the immunomodulation, which contributes to a hyperactivation of T-lymphocytes and subsequently to an increased release of inflammatory cytokines.

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Toremifene, a triphenylethylene antiestrogen, in the treatment of breast cancer

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The pharmacokinetics and clinical use of a new triphenylethylene antiestrogen developed in Finland, toremifene, was studied in postmenopausal women. The pharmacokinetics was studied in 82 healthy volunteers and 12 women with advanced breast cancer. The efficacy and toxicity of the treatment was investigated in 63 patients with advanced ER-positive breast cancer. The correlations between estrogen and progesterone receptors and treatment results with toremifene were analyzed in a group of 113 women treated for advanced breast cancer.

Toremifene was absorbed completely after peroral administration giving a serum peak concentration at 2–4 h after ingestion; the distribution half-life was about 4 h. The mean elimination half-life was 5 days and patients on continuous daily dosing reached the steady state serum level in 6 weeks. Two metabolites were quantified in serum samples: N-demethyltoremifene was present in high concentration and deaminohydroxytoremifene in a concentration one-tenth of that of unmetabolized toremifene.

Sixty mg of toremifene was safe and effective for treatment of patients with ER-positive breast cancer. The response rate (CR + PR) was 54% (95% confidence interval 39%–69%) and the estimated median duration of response 20 months. No serious side-effects were observed during follow-up which also included monitoring of the immunological status in 12 patients. Indeed, an increase in the response of lymphocytes to mitogen stimulation was recorded, which may reflect a positive effect on cell mediated immunity of the patients.

Twenty mg of toremifene had also antitumor activity. The results were, however, less favorable than with 60 mg: 3 PR in 14 patients resulted in a response rate of 21%, with a 95% confidence interval of 5%–51%. The 20 mg dosage cannot be recommended.

The response rate did not correlate significantly with the ER concentration or the presence of PgR in the tumor tissue. The ER concentration of tumor tissue predicted, in contrast to PgR, independently the duration of response.

Toremifene can be used safely to treat ER-positive breast cancer in postmenopausal women. Administration once daily by mouth can be used. Approximately half of the patients respond to 60 mg of toremifene, irrespective of the concentration of ER or the presence of PgR in the tumor tissue. The efficacy of toremifene in ER-negative breast cancer is unknown.

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Activation of human cytotoxic cells with cytokines—Studies on the tumoricidal capacity in the absence and presence of monoclonal antibodies

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The generation of human cytotoxic cells was studied in vitro with the aim to optimize their production and activation for therapy. Lymphocyte activated killer (LAK) cells could be generated without using Ficoll-Isopaque separation (FIP). The technique reduced production time as well as lowered the cost. Cells generated without FIP separation had a higher expansion index in long-term cultures with IL-2, as compared to the commonly used technique culturing FIP cells for 4 days. It was possible to expand LAK cells at a high cell density for 20 days using serum-free medium. Longer culturing periods increased the total cell numbers of FIP as well as of non-FIP separated cells. The latter population displayed a higher expansion index and an increased total number of $CD3^+$ and $CD56^+$ cells as compared to FIP separated cells.

In order to circumvent the inhibitory effects of monocytes on LAK cell induction, FIP separated cells were pretreated with phenylalanine methylester (phe-O-Me), which reduced the number of monocytes and increased the total lytic capacity after 96 h of culture with IL-2. After 20 days of culture, phe-O-Me did not enhance LAK activity and cells did not grow well.

The non-FIP separated cells displayed an increased cell yield and total cytotoxic capacity against Daudi target cells, as compared to FIP separated cells, both in short-term and long-term cultures. Pretreatment of FIP or non-FIP cells with phe-O-Me was not superior to non-FIP separated cells without phe-O-Me at any culture time. Thus, phe-O-Me pretreatment is preferable only in short-term culture of FIP cells. When using non-FIP cells, phe-O-Me is not recommended.

In order to further enhance the lytic potential of cytotoxic cells, the synergistic activities of other cytokines such as TNF-a, a-IFN, GM-CSF, and IL-4 were investigated in the absence and presence of a mouse monoclonal antibody (MAb 17-1A). TNF-a alone did not induce lymphocytes to kill tumor cells. TNF-a and IL-2 acted synergistically to generate a cytotoxic cell population (IL-2/TNF cells). IL-2/TNF cells could also be produced on a large-scale basis for therapy. Antibody-dependent cellular cytotoxicity (ADCC), using MAb 17-1A against the colon cancer cell line SW-948, was augmented after preactivation of lymphocytes with IL-2. The cytotoxic activity could be further potentiated if a-IFN was present during the cytotoxicity assay. IL-2/TNF cells had a high lytic capability in ADCC as compared to effector cells stimulated with IL-2 alone. GM-CSF has earlier been shown to potentiate ADCC. The combination of IL-2 and GM-CSF synergistically enhanced the lytic activity of lymphocytes and significantly increased the number of $CD25^+$ cells as compared to cells stimulated with IL-2 or GM-CSF alone. IL-4 also augmented ADCC of monocytes and NK-cells as well as the NK activity. Chimeric MAb 17-1A was found to be significantly more effective in tumor cell lysis than the mouse MAb 17-1A.

Cytotoxic cells could be generated from ascitic fluid of patients with ovarian carcinoma after long-term cultures. The effector cells were mainly $CD8^+$, $CD56^{+/-}$ cytotoxic T cells, $CD4^+/CD29^+$, memory T cells, and $CD3^-$, $CD56^{+/-}$ NK cells with augmented expression of adhesion molecules.

The combination of cytokines and monoclonal antibodies seems to be an interesting and promising combination for future biotherapy, especially the combination of IL-2 and GM-CSF together with chimeric antibodies.

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Human papillomavirus and human immunodeficiency virus infection in relation to cervical cancer—Studies and observations of basic clinical and epidemiological aspects of cancer of the cervix with special reference to Kenya, Africa

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Carcinoma of the cervix is the most common female malignancy in Kenya. No screening program has been started in the country and, with only one public hospital with high energy radiation facility, few women receive optimal care. Little is known about the epidemiology of the disease in Kenya although it is clear that smoking and contraceptive use are unlikely to play a major role due to their low prevalence. Analysis of clinical features in 1 210 patients treated in Nairobi between 1978–1984 revealed a mean age of 42 years and frequent high parity. Only 7% presented with stage I disease. Follow-up was extremely poor. In an analysis of knowledge and attitudes, ignorance about the disease and preventive measures was confirmed in both cases and controls. Lack of appropriate information and cultural beliefs described may hamper the successful introduction of a screening program. Analysis of human papillomavirus (HPV) DNA in cervical smears from cytologically normal FP clinic attenders in Nairobi by PCR showed positivity in 19.5%, a figure comparable to current rates in low-risk women in Umeå, Sweden. In squamous cervical cancer biopsies from Kenya, HPV 16 DNA was detected in 85%, HPV 18 in 69% and HPV 6 in only 23%. Double infection with HPV 16 and 18 was common (62%). Using PCR on archival paraffin-embedded biopsies from cervical adenocarcinoma from Sweden, HPV DNA detection rate was 42%; 15% were HPV 16 and 27% HPV 18. This confirmed recent reports of HPV involvement in cervical adenocarcinomas. Human immunodeficiency virus (HIV) seroprevalence in 200 cervical cancer patients in Kenya was only 1.5%, much lower than in known high-risk groups but comparable low-risk groups. The findings and observations highlight the problem of cervical cancer in Kenya which is not dissimilar to other African countries. Critical issues to be overcome if incidence and mortality are to be reduced are discussed. The prevalence of human papillomavirus is analysed and the need for further studies to identify, explore and quantify the importance of specific risk co-factors peculiar to a high prevalence area like Kenya are discussed.

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Monoclonal antibodies identifying the α/β T cell receptor variable domains and two novel cell membrane antigens on lymphocytes

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Monoclonal antibodies (MAb) to the variable domain of the T cell receptor (TCR) were generated. The TCR-MAbs could be grouped into different groups, according to their respective reactivity with peripheral blood lymphocytes from blood donors. These reactivity patterns probably represent a range from public to private epitopes on the variable domain of the TCR.

An ELISA and subcloning method for the selection of hybridomas secreting murine Ig2a subclass spontaneous switch variants was established.

Two novel lymphocyte cell membrane antigens were identified and characterized by the MAbs designated H2 and IVF7. The MAb IVF7 reacted with a 38–40 kD antigen (reduced), mainly expressed on the majority of NK cells and a minority of T and B cells, suggesting a relation to the cytotoxic capacity of these cells. MAb H2 reacted with an 80 kD antigen, weakly expressed on the majority of resting T cells and a minority of B cells.

One T cell chronic lymphatic leukemia (T-CLL) patient was treated with a TCR-MAb. An 80% tumor reduction was seen after 2 mg of administered TCR-MAb. In vitro cytotoxic tests (antibody-dependent cellular cytotoxicity or complement) were negative, suggesting more complex mechanisms for tumor elimination. The side-effects of the administered MAb were mild and likely related to lymphokine release. The results indicate that TCR-MAbs could be an effective therapeutic modality in T cell malignancies.

In the peripheral blood a skewed reactivity between the $CD4^+$ and the $CD8^+$ T cell populations of some V gene segment TCR-MAbs was seen. A similar skewedness was also present on umbilical cord blood T lymphocytes and thymocytes, suggesting that a positive selection mechanism in the thymus regulates the respective $CD4^+$ and $CD8^+$ T cell repertoires.

A predominant usage of V gene segments in T cells could be demonstrated in patients with multiple myeloma and monoclonal gammopathy of uncertain significance (MGUS), indicating a clonal expansion of T cells. The functional relation of these T cell populations to B cell disease is still unknown. However, if such interaction exists, new therapeutic possibilities arise.

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