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.

Carcinoid heart disease—A clinical, biochemical and morphological study

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It is unique for a malignant tumor to be associated with a pathognomonic cardiac disease which is an important cause of morbidity and mortality. Malignant mid-gut carcinoid tumors are causally related to characteristic lesions or plaques typically located on the mural and valvular endocardium of the right side of the heart, constituting carcinoid heart disease.

In the present study it was found that the cardiac lesions not infrequently infiltrated through the endocardium and lamina elastica interna into the myocardium. The lesions consisted of an elastin-deficient stroma rich in acid mucopolysaccharides and collagen intermingled with a small to moderate number of cells. The cells had immunohistochemical characteristics of muscle cells and showed a low proliferation rate.

Transthoracic cardiac ultrasound examinations revealed signs of carcinoid heart disease in about 70% of consecutively investigated patients with malignant mid-gut carcinoid tumors. The most frequent pathological findings were morphological and functional abnormalities of the tricuspid valve and increased right heart cavities. Transesophageal echocardiography demonstrated more accurately the morphological changes of the tricuspid valve and the right atrium and seemed to visualize the endocardial myofibromatous lesions observed histologically.

Patients with carcinoid heart disease and right ventricular failure had significantly higher plasma levels of atrial natriuretic peptide (ANP) than those with fewer or no signs of carcinoid heart disease or controls. These levels increased in parallel with clinical signs of right ventricular failure and declined and normalized when right ventricular failure could be successfully managed by valvular surgery.

Reconstructive valvular surgery was of obvious beneficial value in patients with advanced carcinoid heart disease and right ventricular failure. Serial determinations of plasma ANP seemed to offer guidance for the timing of surgery.

The etiology of carcinoid heart disease remains obscure, but tumor-released vasoactive substances might be involved in the pathogenesis.

May 1989

Acute lymphoblastic leukaemia—Studies on prognosis and effects of treatment with special reference to adults

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Acute lymphoblastic leukaemia comprises a heterogeneous group of haematological malignancies. It was shown in the present work that routine morphological examination often is insufficient for a proper diagnosis and hence must be supplemented with cytochemical tests and immunological characterization of the leukaemic cells.

It is concluded that the number of patients with non-responding leukaemia decreases and the probability of a prolonged duration of remission increases with intensive remission induction therapy including both an anthracycline and L-asparaginase is used.

Data are presented on patients in their 2nd and 3rd complete remission of ALL which show that a substantial number of patients achieved a longer remission after autologous bone marrow transplantation (ABMT) compared with their previous remission. This indicates that ABMT is beneficial. Ex vivo purging of harvested bone marrow with monoclonal antibodies and complement, in connection with ABMT, has no adverse effect on haematological reconstitution.

In the present studies it was found that the frequencies of $CD10^+$ mononuclear cells in bone marrow and peripheral blood increase during normal regeneration after ABMT, hence CD10 has no value as a single marker in remission diagnostics. On the other hand, $CD10^+TdT^+$ cells are rarely found in the blood ($< 0.03\%$) in remission, but increase and sometimes herald morphological relapse in patients with $CD10^+$ leukaemia.

The granulocyte proteins myeloperoxidase, lysozyme and lactoferrin were found to reflect the bone marrow activity and herald the return of neutrophil granulocytes into the blood by 4–10 days after ABMT.

Allogeneic bone marrow transplantation (BMT) is known to impair several endocrine functions, but very little data have been presented after ABMT. Impaired glucose metabolism was observed 6 months after ABMT as investigated with an intravenous glucose tolerance test.

In summary, a substantial number of adult ALL patients can be cured with an intensive treatment protocol. Patients at high risk of relapse should be offered BMT or ABMT in controlled trials, where acute and long-term toxicity as well as quality of life should be compared between different treatment modalities.

May 1989

Salivary gland tumorigenesis—A cytogenetic and molecular study

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The general aims of this investigation were to study the role of chromosome rearrangements in the genesis of human and experimental salivary gland tumors, and the relationships between specific chromosome rearrangements and different genes involved in neoplasia.

In a series of 56 benign human pleomorphic adenomas it was shown that the frequency of abnormal stemlines could be increased from about 50% to about 80% by modifications of the tissue culture technique. Tumors with abnormal stemlines were characterized by, in particular, reciprocal translocations involving the chromosome regions 3p21, 8q12 and 12q13–15. A t(3;8)(p21;q12) was seen in no less than 9 tumors. The breakpoints on chromosomes 3, 8 and 12 coincide with the location of oncogenes and fragile sites. It is worthy of notice that 71% of the abnormal stemlines showed a proximal long arm rearrangement of No. 8 involving the same band as the *c-mos* gene maps do.

The chromosomal patterns in 29 malignant salivary gland tumors differed markedly from that observed in pleomorphic adenomas. The most consistent rearrangements seen among the malignant tumors were deletions, or rarely translocations, involving the long arm of chromosome 6. The breakpoints in the

majority of tumors clustered to bands 6q22–25. Other recurrent deviations were loss of the Y chromosome or trisomy 8. The deletions were found in all major types of malignant salivary gland tumors, indicating that a common pathogenetic mechanism for these tumors might well be loss of a tumor suppressor gene located at 6q25-qter.

Using immunohistochemical and immunoblotting techniques the expression of *ras* oncogenes was studied in both benign and malignant salivary gland tumors. Pleomorphic adenomas were found to express high *ras* p21 levels, whereas malignant salivary gland tumors either did not express the p21 protein, or expressed lower levels. A novel correlation between high *ras* p21 expression and pleomorphic adenomas with chromosome 8 rearrangements was found, suggesting the presence of *ras* regulatory sequences on the proximal long arm of chromosome 8, at band q12.

Polyoma virus-induced malignant salivary gland tumors in CFLP-mice showed an extensive karyotypic heterogeneity. Although clonal structural and numerical deviations were found in all tumors, there was no deviation that was common to all tumors. By in situ hybridization a major polyoma viral integration site was mapped to chromosome 14, band B. It is concluded the chromosomal instability plays an important role in the early evolution of polyoma virus-induced salivary gland tumors.

In summary, this investigation shows that benign and malignant salivary gland tumors constitute a very useful model system for detailed molecular studies of the relationships between chromosomal translocations and deletions and different genes involved in neoplasia, in particular oncogenes and tumor suppressor genes.

May 1989

Chronic lymphocytic leukemia of B cell type and monoclonal B cell lymphocytosis of undetermined significance—A study of the B cell clone and regulatory lymphocyte subpopulation

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The B cell clone and T and NK cell populations were studied in patients with chronic lymphocytic leukemia of B cell type (B-CLL). The term 'Monoclonal lymphocytosis of undetermined significance (B-MLUS)' was introduced for patients with a clinically benign condition and a monoclonal B lymphocytosis in peripheral blood and bone marrow but without other signs or symptoms of disease.

The surface immunoglobulin (sIg) isotype expression on the leukemic cells was of μ or $\mu\delta$ type in the majority of patients. A μ^+ B cell clone was found predominantly in patients with progressive disease while patients with indolent disease had μ^+ δ^+ leukemic cells. Fifteen per cent of the patients had a sIg^- B cell clone expressing surface markers consistent with various stages of intermediate B cell differentiation. Six out of 8 patients with more than 1.5% γ^+ cells had IgG restricted to one subclass.

The numbers of S $phase^+$ cells were higher in the μ compared to the $\mu\delta$ group ($p<0.001$) and the surface expression of receptors for growth factors (CD23, CD25 and CD71) was higher in patients with progressive B-CLL compared to D-MLUS patients.

Patients with a prominent lymphadenopathy had $CD22^+$ leukemic cells while in patients with progressive lymphocytosis without lymph node enlargement the B cell clone expressed Leu8. This might indicate an association between these surface antigens and homing capacity.

The clinical value of characteristics of the B cell clone was confirmed in Cox multivariate analyses where a μ^+ leukemic clone was a predictor of short survival and high numbers of S $phase^+$ cells were associated with a poor prognosis.

Patients with progressive B-CLL had higher total numbers of $CD8^+$ and $CD57^+$ cells and a lower CD4/CD8 ratio than B-MLUS patients and control donors. A high proportion of $CD16^+/CD57^-$ cells (a population with high NK activity) was found mainly in B-MLUS patients.

B-MLUS and progressive B-CLL might represent the two extremes of a spectrum of chronic leukemic B cell disorders. The variability in T and NK cell subpopulations might reflect differences in the clinical course.

May 1989

Differentiation inducing factor, tumor necrosis factor, and lymphotoxin–Cytokines with effects on growth and differentiation of myeloid leukemic cells

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In leukemia the regulation of growth and differentiation is deranged. A main characteristic of leukemic cells is their unability to terminally differentiate. As a result, they escape from normal growth control and give rise to a clinically overt leukemia. The aim of this thesis was to extend the knowledge on differentiation in leukemic cells. The cytokines differentiation inducing factor (DIF), tumor necrosis factor (TNF), and lymphotoxin (LT) were found to be tentative inducers of differentiation. Their effects on hematopoietic cells in vitro and their molecular mechanisms of action were investigated.

Similar growth inhibitory and differentiation inducing effects were found for all 3 cytokines. Among cell lines a varying sensitivity was found; while some cell lines were growth inhibited at picomolar concentrations of the cytokines, others were resistant at these concentrations but responded with terminal differentiation at 10-fold to 100-fold higher concentrations of cytokines. Also fresh normal and leukemic hematopoietic cells were sensitive to an antiproliferative effect. A difference in susceptibility to LT was seen between normal myeloid progenitor cells and cells from patients with chronic myeloid leukemia (CML); normal cells were growth stimulated at low concentrations of LT while CML-cells showed to be highly susceptible for the antiproliferative effect of LT.

DIF, TNF, and LT were shown to share common binding sites on the target cells. The receptor system was characterized. A single class of high affinity receptors (K_D 150–300 pmoles/l) with a density of 1 500–3 000 per cell were found. No correlation between number of receptors or affinity and biological sensitivity was seen. After binding to cell surface receptors the cytokines were internalized through receptor mediated endocytosis and transferred to lysosomes where both ligand and receptor were degraded. A spontaneous internalization of receptors was observed also in the absence of ligand. The maintenance of a constant expression of receptor molecules was dependent on de novo protein synthesis. The molecular weight of the receptor was determined to 70 kD. Asparagine-linked carbohydrates constituted 4–5 kD of the molecular weight.

May 1989

RES macrophage function and tumour growth—Methods of measurement, experimental studies and monoclonal antibody kinetics in the rat

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The reticuloendothelial system (RES), or monocyte phagocyte system (MPS), is composed of cells throughout the body in close contact with the circulatory system. These cells line liver sinusoids, lymphducts and alveoli can, by phagocytosis, eliminate foreign particulate matter. Macrophages also present antigens to lymphocytes and secrete important biological response modifiers.

This study evaluates dynamic liver RES scintigraphy for registration of the functional level of the RES or monocyte phagocyte system. Tumour growth and tumour take in the liver and in the kidney were examined in relation to RES activity. Dynamic liver RES scintigraphy was employed in evaluation of the host defence in liver tumour-bearing rats treated with RES-stimulating Zymosan. The kinetics of monoclonal antibodies raised against cancer antigens were studied in normal and tumour-bearing rats. The rats underwent stimulation or depression of the RES.

Three technetium-labelled colloids, Albures, Nanocoll and sulphur colloids of different sizes and amounts, were investigated for use in RES function assessment in normal Wistar rats. The colloids were injected i.v. in anaesthetized rats. Dynamic recordings with a gamma-camera were made over the liver and heart and consecutive blood samples drawn. Clearance rate constant is described according to the slope of the plot ln [1- U(t)/Ufinal] versus t, where t is time and U count rate in the region of interest (ROI) on consecutive gamma-camera images of the rats after Tc-colloid injection. Normal Wistar rats and Zymosan RES-stimulated rats were examined with colloid amounts above and below the 'critical colloid dose' to investigate conditions for registering RES macrophage activation. Blood-flow changes were recorded with the ^{133}Xenon wash-out technique in the liver. A nitrosoguandine (NGW)-induced syngenic colonic adenocarcinoma was inoculated into the liver and the kidney of normal, Zymosan (3 mg/100 g) RES-stimulated and methyl palmitate (100 mg/100 g) RES-depressed rats to investigate RES influence on tumour take and tumour growth in a macrophage-rich organ like the liver or macrophage-poor organ like the kidney. NGW tumour cells were inoculated into the liver and repeated measurements of RES function with dynamic liver RES scintigraphy were done on controls, tumour-bearing rats and rats pretreated with RES-stimulation by Zymosan (3 mg/100 g). Monoclonal antibodies (MAb) raised against sialyted Lewis^a cancer-associated antigen were used in a tumour model with no measurable antigen in the blood in order to study MAb kinetics in tumour-bearing and RES-modulated rats. RES was stimulated with Zymosan (3 mg/100 g) and depressed with methyl palmitate (100 mg/100 g)

There was good a correlation between blood sample–heart blood disappearance curves and liver uptake registrations. Albures and sulphur colloids could, for hemodynamic reasons, not be given in amounts that lead to decreased clearance rate. Nanocoll could be given in amounts above the 'critical colloid dose' and a decrease of clearance rate was registered. In RES-stimulated rats, Nanocoll could be used to register the increased clearance rate, but the ratio between Nanocoll and Albures clearance rates remained the same, indicating no changes in extraction in the whole rat but in blood flow to a proliferated RES organ. An in vivo registration revealed increased extraction in the liver. In RES-stimulated animals, a significant decrease of tumour take in the macrophage rich liver could be registered. RES stimulation or depression had no significant influence on tumour take or tumour growth in the kidney.

Dynamic liver RES scintigraphy could register increased clearance rate in rats, not only after Zymosan RES-stimulation but also after tumour cell inoculation in the liver. RES-stimulation led to decreased tumour size in the liver and prolonged survival after tumour inoculation. A weak correlation between survival and RES clearance rate was found. The kinetics of iodinated MAb against tumour-associated antigens were not influenced by RES host modulation, indicating an unspecific action of RES macrophages on elimination of MAb. Tumour growth in the host led to increased elimination of MAb.

May 1989

Prognostic and therapeutic indications of DNA content in squamous cell carcinomas of the upper respiratory-digestive tract

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Malignancy grading using Jakobsson's scale (1973) gave prognostic information in lip and oral cavity carcinomas, and its clinical use is recommended. However, the pathologist should have special experience of squamous cell carcinoma of the head and neck.

The preparation method described by Hedley and co-workers (1983) was tested on breast carcinomas for comparing fresh and formalin-fixed tissue. There was good correlation concerning DNA ploidy and S-phase estimation. The use of formalin-fixed, paraffin-embedded specimens allows retrospective studies of patients in whom the clinical outcome is known. The study also shows that the results are reproducible and that the preparation method yields enough cells for measurements from small lesions, which is important when analysing for example small laryngeal tumours.

Preparations from squamous cell carcinoma yielded good results with close correlation between DNA ploidy and S-phase values in fresh and embedded specimens. The Hedley method is thus appropriate in squamous cell carcinomas.

DNA ploidy and S-phase value yielded no statistically significant differences in prognosis between different groups of lip, laryngeal, or oral cavity carcinomas. However, DNA polyploid cell nuclei in lip carcinomas were associated with a worse prognosis, and the same seemed to apply to laryngeal and oral cavity carcinomas. This problem could be clarified by further and more extensive studies. A major difficulty with head and neck carcinomas is the fact that so few patients are seen at individual hospital centres. Multicentre studies are therefore necessary to obtain material of an adequate size before any conclusions concerning the prognostic value of DNA content in squamous cell carcinomas are drawn.

An important potential application of DNA estimation is to compare DNA ploidy and S-phase value with the response to radiotherapy; this could broaden the understanding of tumour biology. The present study shows that DNA aneuploid tumours and tumours with a high proliferative activity seem to be the most radiosensitive.

Static or flow cytometry can be used to assess DNA content in premalignant lesions. DNA abnormalities are characteristic of some premalignant lesions (Klein et al., 1982). The small size and focal nature of premalignant lesions makes the deparaffinized block method well suited for such studies. DNA analysis may thus provide a complement for the pathologist.

Another application of the method is in determining whether a tumour is primary or metastatic. The presence of identical DNA aneuploid values in 2 synchronous or metachronous tumours in

the same organ system is strong evidence that one of the tumours is metastatic (Barlogie et al., 1983).

In addition to DNA content, factors such as nuclear antigen (Bauer et al., 1986) and cell size (Stål & Hatschek, 1988) can prove useful to characterize squamous cell carcinomas, and to separate DNA diploid tumour cells, normal cells, and lymphocytes, and possibly also to solve the problem of classifying tumours into different DNA ploidy groups.

May 1989

Reproductive factors and risk of cancer of the breast and genital organs—A prospective study of Norwegian women

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The following main conclusions may be drawn from the results of the present follow-up study:

Cancer of the breast, corpus uteri and ovary

1. A history of many full-term pregnancies was associated with low risk of cancer of the breast, corpus uteri and ovary. A high number of incomplete pregnancies may also be associated with low risk of these cancers, but the observed trends were weak, reaching statistical significance only for breast cancer. Among nulliparous women, the ever-married did not seem not to be at increased risk, neither for endometrial, ovarian or breast cancer, compared to never-married women. This observation as well as the results showing no clear trend for married women according to duration between marriage and first delivery for any of these cancers, indicate that subfertile women may not be at increased risk for developing these cancers in higher age groups.
2. Age at first and last birth were positively associated with risk of breast cancer and inversely associated with endometrial cancer, whereas no clear associations were observed with ovarian cancer. This may indicate that the effect of a pregnancy is mediated, at least in part, by different mechanisms for these three cancers.
3. Age at menarche was inversely and age at menopause directly related to risk of breast and endometrial cancer, whereas no association was observed for ovarian cancer. The effects of age at menarche and age at menopause seemed to be stronger for endometrial than for breast cancer. These findings may point to a difference in effect of ovarian hormones on the risk of these cancers.
4. For cancers of the breast, endometrium and ovary the protective effect of high parity seemed to be of lifelong duration. The associations with number of full-term pregnancies and age when pregnancies occur seemed to differ according to age at diagnosis for breast cancer, whereas no similar effect modification was noted for endometrial or ovarian cancer. For cancers of the breast and endometrium, late age at menarche seemed to protect until old age. The effect of age at menopause lasted to old age and was strongest in the age group of 80 years or more for breast cancer, whereas for endometrial cancer this effect was not strong after the age of 70.
5. Mean duration of lactation for each child or total duration of lactation were not significantly associated with any of these cancers in analyses with adjustment for parity. For breast cancer a nonlinear relation was indicated, with low risk both among women reporting very short lactation and women reporting long mean duration or long duration for any of the three first children. These findings might indicate that physical factors related to lactation can influence breast cancer risk.
6. With the possible exceptions of Paget's disease of the breast and mucinous cystadenocarcinomas of the ovary, the reproductive factors seemed to affect different histological subtypes of the different sites similarly. However, for a safer assessment of specificity in effect according to histological type, larger series grouped by the use of a uniform system of classification will be necessary.

Cancer of the cervix uteri

The associations observed with reproductive factors were generally expected from the known relationships between sexual habits and the risk of squamous cell carcinoma of the cervix uteri. High risk was observed in married women, especially among those married more than once. The high risk observed in women of high parity was accounted for by confounding with age at first birth, which was strongly and inversely associated with risk, in analyses with adjustment for parity and some other potential confounders. The strong association with age at first birth might suggest that pregancy-related factors other than sexual habits may influence risk. Age at menarche, age at menopause or duration of lactation were not significantly associated with squamous cell carcinoma, which suggests that hormonal factors are not involved in the etiology. The associations observed with adenocarcinomas of the cervix uteri indicate that the two histological types are differently affected by reproductive factors.

Other cancer sites

The reproductive factors that showed the strongest associations with cancers of the breast and genital organs were not similarly related to the total risk of cancers of all other sites. This suggests that the associations observed are specific to cancers of the breast and genital organs.

May 1989

Molecular abnormalities of chromosome 7 in myeloid disorders

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Monosomy 7 and partial deletion of chromosome 7 long arm are common recurrent chromosome abnormalities in human acute nonlymphocytic leukemias and myelodysplastic syndromes. Altogether 24 patients were studied by applying chromosome 7 specific probes and molecular techniques to characterize chromosome 7 abnormalities. Differentiation of white blood cells was studied by using the loss of heterozygosity for chromosome 7 loci as a marker of affected and unaffected lineages. Submicroscopic deletions and molecular rearrangements were sought by comparing probably affected and unaffected lineages in patients with two apparently normal chromosomes 7. Chromosome 7 long arm deletions were mapped in molecular terms by using molecular probes with known locations.

The conclusions were:

1. Chromosome 7 abnormalities could be detected by comparing restriction fragment length polymorphism analysis results of fractionated white blood cells. Lymphocytes were found to be unaffected by monosomy in all patients, whereas granulocytes and/or monocytes were monosomic.

2. Granulocytes and monocytes were differently affected in two patients with myelodysplastic syndrome and monosomy 7. Heterogeneity of differentiation pathways was suggested as an explanation complementary to the heterogeneity of stem cell level involvement in myelodysplastic syndrome.
3. Several genes and genomic sequences were found to be deleted in four patients with deletions of the chromosome 7 long arm, including the MET proto-oncogene, the plasminogen activator inhibitor type I gene, and the multi-drug resistance gene linked sequence MDR2. The proalpha2(I)collagen and erythropoietin genes remained undeleted.
4. The occurrence of interstitial deletions was confirmed by the detection of undeleted terminal sequences in one patient.
5. Submicroscopic deletions or gene rearrangements were not detected in patients with two apparently normal chromosomes 7.
6. In all four patients with a deletion of chromosome 7 the proximal breakpoint mapped between two closely linked genes in band q22, suggesting a specific preferential breakpoint site. In one of two patients studied, the breakpoint was provisionally mapped within 195 kilobases of the erythropoietin gene. The gene order centromere—proalpha2(I)collagen–erythropoietin–breakpoint–telomere was suggested.

May 1989

Gene expression during megakaryoblastoid differentiation of K562 leukemia cells

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The K562 leukemia cell line, derived from a patient with chronic myeloid leukemia (CML) in blast crisis, contains a chromosomal rearrangement, which results in the expression of a novel fusion-protein, the tyrosine kinase p210. p210 contains an activated *abl* oncoprotein and is typical for CML. Treatment of K562 cells with the tumor promoter 12-0-tetradecanoylphorbol-13-acetate (TPA) led to an arrest in their growth and a concomitant inhibition of the activity of the p210 tyrosine kinase, although the corresponding mRNA level did not change. In the same experiments no changes were observed in $p60^{c\text{-}src}$ tyrosine kinase activity. This suggests that p210 synthesis was down-regulated at the translational level or that a phosphatase or an inhibitor of p210 activity was induced in the TPA-treated cells.

K562 cells cultivated in the presence of TPA acquired megakaryoblastoid characteristics as assessed by the appearance of surface antigens typical for platelets. The regulation of mRNAs coding for platelet derived growth factor (PDGF) A- and B-chains, and transforming growth factor β1 (TGFβ1), and the biosynthesis of the corresponding proteins were analysed in the differentiating cells. During megakaryoblastoid differentiation induced by TPA in the K562 cells, the mRNAs for both the A- and B-chain genes of PDGF accumulated in the cells, whereas induced erythroid differentiation was accompanied by accumulation of only B-chain mRNA. The genes coding for the A- and B-chains of PDGF are thus differentially regulated and co-ordinately expressed in K562 cells during their megakaryoblastoid, but not erythroid, differentiation, when the cells also synthesize and secrete PDGF polypeptides. An increased expression of TGFβ1 was also observed in the differentiating cells.

Platelets store and release an inhibitor of fibrinolysis, the plasminogen activator inhibitor type-1 (PAI-1). The expression of PAI-1 was studied at the mRNA, antigen and activity levels including an analysis of its interactions with the urokinase-type plasminogen activator (u-PA) in K562 cell cultures. Induced megakaryoblastoid differentiation of K562 cells leads to enhanced accumulation of the mRNAs for PAI-1 and both u-PA and tissue-type plasminogen activators (t-PA). Enhanced production and secretion of u-PA proenzyme (scu-PA) was followed by its activation and subsequent inhibition by a large excess of secreted PAI-1. A clear difference was found in u-PA activation between cultures of several leukemia cell lines and solid tumor cell lines. More than 70% of the u-PA in cultures of leukemia cells was in the active form (tcu-PA), whereas in cultures of solid tumors over 80% was in the inactive proenzyme form (scu-PA).

May 1989

Characterization of a transplantable rat Leydig cell tumour with reference to plasma membrane receptors, adenylate cyclase activity, testosterone secretion, tumour growth and morphology

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The investigated transplantable rat Leydig cell tumour (H-540), which originally arose spontaneously in a male Fischer rat, can easily be transplanted into male and female Sprague-Dawley rats.

The tumour grows in intact, castrated as well as in hypophysectomized rats. The rate of tumour growth is dependent of the pituitary gland.

The tumour Leydig cells possess receptors for LH/hCG, prolactin, PGE and β-adrenergic hormones. The properties of the LH/hCG and prolactin receptors are identical to those of normal adult rat Leydig cells. The number of LH/hCG receptors is much lower (ca. 1%) than that found in normal Leydig cells, whereas that of prolactin receptors in the tumour Leydig cells is in the normal range. The levels of LH/hCG and prolactin receptors are influenced by pituitary hormones.

The AC of the tumour Leydig cells can be activated by LH/hCG, PGs of the A, E, F and I series, catecholamines and glucagon. Highest activation of AC was achieved with PGE_1.

The PGE_1 and catecholamine responsive AC is regulated by nucleotides and Mg^{2+} slightly different from that described in most other cells.

The Leydig tumour cells produce androgens. Testosterone secretion in vivo increases gradually with increasing tumour size up to approximately 10–15 g in both intact and castrated rats. Plasma levels of testosterone in hypophysectomized rats with tumours were much higher than in tumour bearing intact and castrated rats.

Testosterone secretion in vitro could be stimulated by hCG, PGE_1 and isoproterenol. After prolonged incubations, PGE_1 appeared to be the most potent activator of testosterone formation.

The tumours have not changed their functional or morphologic properties to any significance through multiple transfer generations.

This Leydig cell tumour is a useful tool in the study of different aspects of Leydig cell physiology in vivo and in vitro when great quantitites of Leydig cells and/or purity from other testicular cells are necessary.

June 1989

Monitoring and cancer risk assessment of carcinogens, particularly alkenes in urban air

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An ultimate purpose of the work has been dose monitoring and risk assessment of cancer initiators, particularly in urban air pollution. Monitoring methods, based on hemoglobin adducts, had to be improved with respect to sensitivity, rate of analysis and possibility of identifying unknown adducts.

A method that fulfilled these requirements was developed. This method was based on specific cleavage of alkylated N-terminal valines in hemoglobin.

The method was tested and evaluated partly in comparison with other methods on samples from persons with occupational exposure to ethylene oxide.

During the course of the work it was observed that the valine adducts introduced by ethylene oxide could be formed during storage of blood samples. This artefact formation was studied and a protocol avoiding the disturbance has been suggested.

The method was applied to monitor doses of ethylene oxide and propylene oxide, originating from ethene and propene respectively in animals exposed to gasoline and diesel exhausts, in cigarette smorkers and in persons occupationally exposed to ethene. Knowingly unexposed persons exhibited background levels of adducts probably originating from ethylene oxide and its precursor, ethene.

These background levels were high compared with increments expected from moderate levels of ethene in urban air and environmental tobacco smoke, which could therefore not be monitored in individuals.

The collective cancer risk in Sweden from ethene in urban air was estimated at some 200 cases annually. To the extent that the background levels are caused by ethene/ethylene oxide the associated risk is estimated to be appreciably larger. In animal experiments unsaturatedness of dietary fat and intestinal bacteria were shown to contribute to the background.

The method was applied for the determination of adducts without connection with known exposures. I.a. methylvaline in hemoglobin, probably originating from *S*-adenosylmethionine, was measured.

June 1989

Myeloperoxidase deficient polymorphonuclear leucocytes in leukaemia and allied disorders

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This thesis is a survey of nine previously published articles on MPO deficient PMN. The incidences in leukameia and allied disorders of the presence of this abnormal subpopulation of mature neutrophils and the relationship to clinical course in AML, susceptibility to infections in AML, FAB classification in AML and MDS, cytogenetically defined aberrations in MDS and morphometrical characteristics were investigated. The aims of the studies were to examine the diagnostic as well as the prognostic value of the parameter, to examine the usefulness of the parameter as an predictive indicator of CR and relapse in AML and to examine the concept that MPO deficient PMN may originate from leukaemic precursors.

MPO deficient PMN were found to occur in a minor number (<4% of the total number of PMN) in normal humans and the incidences of an abnormal number (>4%) were found to be about 40% in AML (I, II, III, IV, VIII), 60% in CML (I, VII), 30% in MPD other than CML (VII) and 30% in MDS (V). The highest incidences in AML were found in the FAB subtypes possessing the most myeloid differentiation potential i.e. FAB M2 and FAB M4 (IV). In ALL, CLL, HCL, Hodgkin's disease, anaemia not related to leukaemia and leukaemoid reactions the incidences all were 0% (I, unpublished data). The abnormal MPO deficient PMN subpopulation, if present, disappeared when CR was achieved and reappeared when relapse eventually was developed (II, VIII). In both situations serial determinations showed that the change occurred before the usual routine blood examinations predicted CR and relapse; several days and several months prior respectively (VIII). The probability of obtaining CR was lower in the AML patients with the abnormal subpopulation and the risk of developing relapse higher than in AML patients without the anomaly (II, VIII).

These differences were not statistically significant, however. AML patients, showing an increased number of MPO deficient PMN, revealed a statistically significant increased susceptibility to infections ($p<0.01$) during the preremission phase accounting for 18% to 67% of the total number of infections in this period (III). This increase was positively correlated to the extent of the anomaly ($p<0.002$). The spontaneous occurrence of a subpopulation of MPO deficient PMN in MDS went together with a simultaneous progression in cytogenetically determined clonal chromosomal aberrations and were related to progression in FAB subtype as well (VI). Morphometrically MPO deficient PMN were characterized by a decreased total cell size and an increased nucleus size of the projected images (IX). The differences to normal PMN were statistically significant ($p<0.01$) and the resulting highly increased nucleus to cytoplasm ratio is a striking resemblance to cells in 'malignant' tumours.

In conclusion the presence of a subpopulation of MPO deficient PMN is a diagnostic valuable parameter indicating some sort of proliferative myelogenous disorder and in acute leukaemia suggesting not only AML, but restricting the diagnosis to one of the subtypes with the most differentiation potential, especially FAB M2 and FAB M4. In AML the presence of this abnormal subpopulation is an unfavorable prognostic parameter in respect to susceptibility to infections and probably also in respect to probability of obtaining CR and developing relapse. The parameter furthermore may be a valuable predictor indicating early CR or threatening relapse in AML, which may prove to be of practical importance in the future. Finally these results, and the characterization of the abnormal cells as having an increased nucleus to cytoplasm ratio, viewed together with the fact that others have proved that leukaemic blasts may be induced to differentiate in culture, strongly support the concept that the MPO deficient PMN may be part of the leukaemic process and may be derived from clones of leukaemic progenitor cells.

June 1989

Studies on the pharmacological properties of the toxic proteins abrin and ricin and on the in vitro activity of their conjugates with an antimelanoma antibody

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Progress in the field of IT research will probably occur along different lines. An interesting development is to couple the toxins to growth factors (Raso, 1988) and to hormones (Oeltmann and Wiley, 1988) rather than to MoAbs to achieve cell-specific activity.

The advances in recombinant DNA technology will probably have considerable impact on the IT field. Toxins produced by gene technology are homogenous proteins devoid of sugars and can be modified by site specific mutagenesis. In toxins where the entry function and binding function are separated, it may be possible to delete at the gene level the binding domain and other unnecessary portions of the protein and create ITs that are both highly toxic and specific. Also, it is a possibility to construct fusion proteins where the toxin and the protein conferring specificity are joined at the gene level, as has already been demonstrated (Murphy et al., 1986).

The use of ITs in the therapy of cancer is still in its infancy. The many new imaginative approaches give reason for cautious optimism. However, the translation of new knowledge and technology into improvements in the therapy of cancer takes longer time than generally appreciated and Vitetta et al. (1987) believe it may take 5 to 10 more years to delineate the potentials and limitations of IT therapy.

The pharmocological properties of the toxic proteins abrin and ricin were studied in mice and humans (I) with a view to their possible use in the treatment of cancer, both as native toxins and as part of immunotoxins, ITs.

In both mice and in cancer patients, injected ricin disappeared from plasma according to first order kinetics. In humans the half life was about 2.5 h. Differences in sensitivity to ricin were observed among three strains of mice, high sensitivity being associated with accumulation of higher ricin concentrations in liver, spleen and kidneys and with a more rapid plasma clearance (I).

A phase I trial involving 54 patients with advanced therapy-resistant cancer (II) showed that ricin could be given in doses giving plasma level twice those achieved in mice given therapeutically effective ricin doses. The flu-like toxic symptoms were tolerable and the myelopoiesis was not depressed. One partial response occurred, while stable disease after previous progression was seen in several patients. One patient had a mild allergic reaction.

In is concluded (II) that the toxic symptoms of ricin do not preclude therapeutic use of ricin in humans and that its unique mechanism of action, lack of myelosuppressive activity and demonstrated antitumor activity in animal models warrant a phase II trial.

Antibody formation against abrin and ricin was demonstrated both in mice and humans after repeated injections of the toxins (III). Circulating antigen-antibody complexes could be detected in serum from treated mice. The antibody levels reached in cancer patients treated every second week indicated that eventual therapeutic use of the native toxins as single agents may be effective only for 2 to 3 months. The antibody response was inhibited by cyclophosphamide and prednisolone, suggesting that the period of effective use may be considerably prolonged if the toxins are given together with cytostatic agents having immunosuppressive activity.

Immunotoxins of abrin and ricin were prepared by coupling the toxins by a disulfide linkage to the monoclonal antimelanoma antibody 9.2.27. The interaction of the conjugates with 8 human melanoma cell lines and 4 non-target cell lines was studied with the main purpose of elucidating the factors influencing the cytotoxicity and specificity of ITs. To avoid unspecific binding of the ITs to the cell surface via the gal-binding site, molecular species with exposed binding sites were removed by affinity chromatography.

The 8 melanoma cell lines differed dramatically in their sensitivity to the abrin conjugate (IV). The differences in sensitivity did not reflect the level of the antigen density but were associated with concomitant large differences in the sensitivity of the cells to the native toxins. The abrin conjugate was far more cytotoxic than the corresponding ricin conjugate.

Experiments in the presence and absence of lactose showed that the gal-binding site on the B-chain acted intracellularly in somehow facilitating the translocation of the toxin A-chain from intracellular vesicles to the cytosol, but was not involved in binding of the affinity purified IT to the cell surface.

The protective effects of lactose and of blocking the antigen by preincubation with excess specific antibody, differed in the two melanoma cell lines, LOX and FEMX, which have different sensitivities to IT and native abrin.

The data were interpreted to mean that the response of the melanoma cell lines to the IT reflects different abilities to process endocytosed immunotoxin and to translocate the active A-chain to the cytosol. The latter process seems to occur by two alternative mechanisms, one mediated by the antibody, and a second one facilitated by the B-chain. The lactose-binding site seems to contribute to the B-chain facilitated mechanism. The relative significance of the two mechanisms seems to differ in different target cell lines depending on their inherent sensitivities to native abrin which in turn largely reflects the ability of the cells to internalize and process surface bound abrin.

The results emphasize the large individual differences between histologically similar cancer cell lines in their sensitivity to an immunotoxin and the importance in this respect of intrinsic metabolic properties of the cells.

September 1989