# SEMINAR REPORTS

The following seminars were held during the IXth Meeting of the European Association for Cancer Research in Helsinki, Finland in June, 1987:

CELLULAR ONCOGENES AND TRANSFORMING PROTEINS
**Chairman: G.Klein (Sweden)**

CHROMOSOMES, ONCOGENES AND CANCER GENETICS
**Chairman: M. Schwab (F.R.G.)**

VIRAL CARCINOGENESIS
**Chairman: J. Wyke (U.K.)**

GROWTH FACTORS, HORMONES AND CANCER
**Chairman: C.-H. Heldin (Sweden)**

CANCER AND CELL DIFFERENTIATION
**Chairman: K.Hilsson (Sweden)**

TUMOUR PROMOTION AND PROGRESSION
**Chairman: V. Schirrmacher (F.R.G.)**

INVASION AND METASTASIS
**Chairman: K. Dano (Denmark)**

HOST DEFENCE
**Chairman: J.-P. Mach (Switzerland)**

MOLECULAR BASIS AND TARGETING OF CANCER CHEMOTHERAPY
**Chairman: A. Pihl (Norway)**

MONITORING OF CARCINOGENIC EXPOSURE
**Chairman: K. Hemminki (Finland)**

SUSCEPTIBILITY TO CANCER
**Chairman: H. Autrup (Denmark)**

EPIDEMIOLOGICAL APPROACHES TO CARCINOGENESIS
**Chairman: M. Hakama (Finland)**

The following reports summarise many of the major findings and conclusions from the seminar programme:

## CHROMOSOMES, ONCOGENES AND CANCER GENETICS

Reported by: **Manfred Schwab**

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Up to the recent past the possibility of a direct involvement of cellular genes in development of cancer was accepted only with great reservation - at best. There is good indication, though, coming from various directions for a causal relation between abnormal gene expression and development of cancer.

1) Pedigree studies have provided evidence for increased familial incidence of various forms of cancer, and in many instances Mendelian manifestation of certain cancers has been unveiled;

2) Studies of experimental plant and animal systems have reproducibly shown that individuals of the offspring resulting from certain crosses develop tumours;

3) Cytogenetic studies have revealed specific chromosomal abnormalities associated with specific forms of cancer.

The molecular nature of such putative "cancer genes" remained enigmatic for a long time. In only about 1977 was the first candidate for a potential cancer gene identified. RNA tumour viruses (also known as retroviruses) served as the experimental tool. Some retroviruses possess a gene essential for cellular transformation, and the very provocative finding was made that in all cases a cellular homologue exists that is evolutionary conserved among vertebrates, and also sometimes in invertebrates. This cellular homologue was designated proto-oncogene, and today approximately 20 such proto-oncogenes have been identified. The hypothesis was raised that abnormal expression, resulting from structural rearrangements or deregulation, might be a prime player at various levels during the multistage process of tumour development. Subsequently abnormal expression of cellular oncogenes was discovered in many cancers, and the main underlying causes were identified as chromosomal translocation, point mutation, gene fusion, gene amplification, promoter insertion and suppressor elimination. Although it is difficult to provide direct proof for a causal relationship of abnormal oncogene expression in development of cancer, the information pointing to a role of cellular oncogenes in cancer is overwhelming. It is possible that molecular studies of oncogenes will soon produce probes for early diagnosis of various forms of cancer.

Our laboratory is specifically concerned with the contribution DNA amplification has to the development of cancer. In particular, our studies focus on human neuroblastoma.

Neuroblastoma is a childhood tumour, whose cells frequently show cytogenetic evidence for amplified DNA-"double minutes" (DMs) or "homogeneously staining chromosome regions"

(HSRs). By serendipitous screening, a DNA domain derived from the short arm of chromosome 2 was identified to be amplified in all tumours and cell lines derived from neuroblastomas and carrying DMs or HSRs. The core region of this DNA domain is characterized by the presence of a cellular gene N-myc that is related to c-myc in structure, sequence and the protein it encodes. N-myc is one member of a family of genes that have in common two highly conserved nucleotide boxes and are referred to as "myc-box" genes. Amplifications of another "myc-box" gene, L-myc, are frequently found in human small cell lung cancers.

Amplification of N-myc has been detected, with few exceptions, only in advanced stages of neuroblastoma. Stages with amplification have extremely poor prognosis. The estimated progression free survival of patients with the most advanced form of neuroblastoma (stage IV) is roughly 50% in cases where there is a single copy of N-myc, 20% and 0% in cases where there are 3 to 10, or more than 10 copies respectively. These data suggest that amplification of N-myc may contribute to malignant progression of human neuroblastoma.

## VIRAL ONCOGENESIS

Reported by: J. Wyke

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Studies on viral oncogenesis pose three broad questions. (1) Can viruses be implicated in tumorigenesis in man and animals? (2) If so, what is their mechanism of action? (3) What responses to this activity can be called forth by the infected organism and by society? All three questions were tackled to varying degrees in this seminar, as well as in the excellent plenary lecture on papillomaviruses by H. zur Hausen (Heidelberg, F.R.G.).

(1) Echoing zur Hausen's theme, S. Syrjanen (Kuopio, Finland) described attempts to identify human papilloma virus (HPV) DNA in bronchial squamous cell carcinomas by in situ hybridization. Her positive findings, albeit in a low percentage of tumours, illustrated the power of such "molecular epidemiology" but highlighted the problems posed by the heterogeneity of HPV and the undoubted multifactorial nature of the disease. Studies on endogenous human retroviral activity in tumours and embryos (A.Vaheri - Helsinki, Finland) produced other perplexities. Following earlier leads Vaheri's group raised an antiserum against a decapeptide specified by an endogenous retrovirus sequence. This antiserum recognised an interesting Mr 75000 protein that was purified, characterised and identified in certain human embryonic tissues and some tumours, most notably 50% of breast carcinomas. However, it seems unclear whether this antigen is indeed of retroviral origin and, although it appears interesting, its significance will only be revealed by further studies.

(2) Mechanisms of viral oncogenesis were reviewed by J. Wyke (London, U.K.), giving examples of both indirect effects, in which the virus need not infect the tumour lineage, and direct effects. The latter frequently involve oncogene activation, a theme that permeated many other sessions in the congress but was also addressed in this seminar.

A fascinating presentation by R. Nusse (Amsterdam, Netherlands) examined mouse mammary tumour virus, whose pathogenesis involves the insertional activation of proto-oncogenes, collectively called int, that in some cases at least may act in concert or sequentially during the evolution of a tumour. Work from several laboratories is now revealing the potential functions of these int genes, and the approach taken by Nusse's laboratory is to study the Drosophila homologue of the gene int-1. They found that Drosophila int-1 is expressed during the development in the anterior compartments of fly segments and is, in fact, the locus for a number of "wingless" mutations that span the structural domain of the gene product. This seems the first case in which the Drosophila homologue of a proto-oncogene has been identified as a previously studied locus. So far this finding tells us more about Drosophila genetics than about breast cancer, but we can anticipate an early reversal of this situation.

A rare outcome of retroviral insertional activation of proto-oncogenes is the transduction of oncogene sequences by genetic recombination with the virus, generating an acutely oncogenic agent. As J. Neil (Glasgow, Scotland) pointed out, feline leukaemia virus (FeLV) frequently recapitulates these events when inducing T cell lymphosarcomas in cats. Transduction of c-myc by FeLV was reported three years ago but Neil's group now finds a virus that encodes the B chain of the T-cell antigen receptor. This not only hints that another gene involved in cellular signalling is a

potential oncogene but it raises interesting further questions about its putative mode of action in T-cell leukaemogenesis.

K. Cichutek (Berkeley, U.S.A.) looked again at the *ras* oncogenes implicated in neoplasia both by viral transduction and by transfection with tumour DNA. He challenged the conventional view that activation of these genes results from point mutations at certain limited sites, preferring instead to invoke truncation of a previously undetected 5' exon. An animated discussion led to the uneasy consensus that our present ignorance left room for both mechanisms and, indeed, either might well apply in different circumstances.

Adenoviruses, the familiar workhorse of the molecular biologists, were examined by A. Mirza (Essen, F.R.G.), who looked at the regulation of their expression, and S. Kvist (Epalinges, Switzerland). The latter called attention to one way in which viruses might contribute indirectly to tumorigenesis, by affecting the immune response. The adenovirus type 2 E3/19K protein binds to HLA class I antigens, with consequent inhibition of their cell surface expression. This phenomenon has clear implications for the cytotoxic T cell response but it is, of course, a long road to link the effect to virus associated disease.

(3) Kvist's presentation introduced the host immune response to virus infection. In addition, both zur Hausen and Wyke produced evidence for non-immunological host regulation of viral genomes, at the level of transcription, and hypothesized that this might be important in disease processes. An interesting report by Mackett (Manchester, U.K.) went further and tackled ways to immunize hosts against infection by potentially oncogenic retroviruses. He chose to work with Epstein Barr virus and had to justify firstly the effort to produce a vaccine against this agent and secondly his chosen vehicle for immunization, vaccinia virus recombinants. Once over these hurdles he produced promising results using the viral gp 340 membrane antigens as immunogen. Only lack of time curtailed an interesting discussion. In future years it will be valuable to return to this question and also to examine hepatitis B virus and the *trans*-activating retroviruses, important agents that were conspicuous by their absence from this seminar.

## GROWTH FACTORS, HORMONES AND CANCER

Reported by: **Carl-Henrik Heldin**

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The control of cell growth in tissue culture is regulated by proteins that stimulate or inhibit mitogenesis. These growth regulating factors bind to specific cell surface receptors, whereby intracellular pathways leading to stimulation or inhibition of growth are activated. Recent work from several laboratories has revealed that oncogenes may act by subverting the mitogenic pathway of growth factors.

A major growth factor in serum for connective tissue cells is platelet-derived growth factor (PDGF). It is a dimeric molecule consisting of two different polypeptide chains, denoted A and B (Heldin). The B chain gene of PDGF was acquired by the simian sarcoma virus as the v-*sis* oncogene; cell transformation by simian sarcoma virus is caused by overproduction of a PDGF-like growth factor structurally similar to a B chain homodimer. There are furthermore indications that other dimeric variants of PDGF-like growth factors may also have autocrine and paracrine effects, since a human osteosarcoma cell line secretes a factor similar to a PDGF A chain homodimer, and human platelets contain a factor which is probably a heterodimer of one A and one B chain. There are some indications that the different PDGF-like factors have different functional effects.

Transforming growth factors-$\beta$ (TGF-$\beta$) is a growth regulatory protein which, like PDGF, is found at a high concentration in platelets. Although TGF-$\beta$ stimulates growth in soft agar of some cell types, it exerts an inhibitory effect on most cell types. A549 cells are inhibited by TGF-$\beta$ and the inhibition correlates with an increase in mRNA and secretion of urokinase (Kestin-Oja). In normal lung fibroblasts urokinase expression was decreased by TGF-$\beta$. Divergent effects on normal and malignant cells suggest that TGF-$\beta$ may participate in the regulation of the invasive, proteolytically active phenotype of cancer cells. A potent tumour promoting activity of TGF-$\beta$ on methylcholanthrene treated Balb/c 3T3 cells was reported (Hamel).

One growth factor that is of special interest is fibroblast growth factor (FGF) since it stimulates endothelial cell growth and thereby acts as an angiogenic factor. Two homologous 16 to 17 kD polypeptides with similar biological effects have been isolated and named basic and acidic FGF (Böhlen). Certain tumour cells synthesize basic FGF and the growth of some tumours can

be inhibited by antibodies that neutralize the mitogenic activity of basic FGF. The mechanism behind the inhibitory effect of the antibodies might be to interrupt an autocrine loop, or to inhibit a paracrine stimulation of formation of new vessels which is a prerequisite for tumour growth.

Growth factor receptors are often equiped with protein tyrosine kinase activity. Recent cloning of DNA for mouse and human PDGF receptor revealed that the receptor is conserved between the species and that it is a transmembrane protein with an external ligand binding domain and an internal domain with a region homologous with other protein tyrosine kinases (Cleasson-Welsh). The PDGF receptor is synthesized as a 145 kD precursor which carries about ten N-linked oligosaccharide groups. The 145 kD component then undergoes additional post-translational modifications and reaches a final size of 179 kD.

Bombesin-like peptides have been found to be synthesized by certain small cell lung carcinomas, and to have an autocrine role in the stimulation of growth of these cells. By use of anti-phosphotyrosine antibodies it has been possible to demonstrate that the bombesin receptor is associated with protein tyrosine kinase activity (Comoglio); a 115 kD tyrosine phosphorylated component, presumably a part of the bombesin receptor complex, was identified in four bombesin producing cell lines but not in a non-producing cell line.

The fact that protein tyrosine kinase activity has been found to be associated both with growth factor receptors and with many oncogene products, indicate that cell growth is regulated by tyrosine phosphorylation. To understand these regulatory mechanisms, it is important also to study the reverse reactions, i.e. the dephosphorylation of tyrosine phosphorylated proteins. The purification and initial characterization of phosphatase specific for tyrosine phosphorylated proteins from A431 cell membranes was reported (Buetler).

Besides protein tyrosine phosphorylation, stimulation of phosphatidyl-inositol turnover, and induction of specific genes, e.g. c-fos and c-myc, have been identified as early consequences of growth factor receptor interaction. Epidermal growth factor (EGF) differs from other growth factors, like PDGF and mitogenic neurohormones, in that it only weakly stimulates phosphatidyl-inositol turnover (Moolenaar). Many factors induce c-fos, but with different mechanisms; the induction by neurohormones, but not by EGF, appears to be critically dependent on the release of intracellular $Ca^{2+}$. Neurohormones causes an immediate increase in inositol(1,4,5,) trisphosphate which mobilizes $Ca^{2+}$ from internal stores, followed by an increase both in inositoltetrakisphosphate and inositol(1,3,4,)trisphosphate which may also have second messenger functions. In the same cell system EGF induces synthesis of inositolphosphate and inositolbisphosphate, but have hardly any effect on the formation of inositoltrisphosphate and inositol-tetrakisphosphate.

In summary, the presentations at this seminar illustrated the current status in the research on some of the more well known growth regulatory proteins. Several examples were given indicating that these factors, or their receptors, may participate in cell transformation in vitro. An important aim for the near future will be to explore whether subversion of growth regulatory pathways are important for tumour formation in vivo.

## CANCER AND CELL DIFFERENTIATION

Reported by: K. Nilsson

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Growth and differentiation during embryogenesis, and development of bone marrow and epithelial stem cells in adult tissue, are complex processes involving distinct but coupled gene programmes - for expansion and specialization, respectively, of the progeny of the stem cells. The genes involved in proliferation and maturation are regulated by subcutaneous (s.c.) growth and differentiation factors, some of which having been structurally defined, and as yet not fully understood signals evoked by cell-cell contacts. Although the cellular mechanisms for the homeostasis of cellular growth and differentiation have been but very fragmentarily clarified, it appears the two inversely related processes require multiple external signals (growth- and differentiation factors), being recognized by factor specific cell surface receptors and transmitted to the nucleus by several cytoplasmic second messenger pathways. The external signals may be provided from plasma (endocrine; e.g. insulin), released by neighbouring cells (paracrine; e.g. PDGF, Il-2) or produced by the same cells (autocrine; e.g. PDGF, Il-2). The effect of the growth and differentiation factors is concentration and time dependent. The rate of growth is highest early during the differentiation process and will gradually

decrease as maturation proceeds, and terminally differentiated cells eventually become arrested in G0/G1 of the cell cycle. In many cell types this growth arrest is irreversible while in others, e.g. lymphocytes, a second event of growth/maturation may take place only to once again terminate with an arrest in G0/G1.

In haematopoietic malignancy and in cancer the malignant phenotype is almost exceptionally that of non-terminally differentiated cells and it has therefore often been suggested that malignant cells represent clonal expansions of cells irreversibly arrested in differentiation.

During the last decade several in vitro cell models have become available for studies of the malignancy-associated deranged control of growth and differentiation. These models include several types of mouse and human leukaemia and lymphoma, mouse embryonal carcinoma, mouse and human malignant epithelial cells (e.g. lung and breast cancer) and neuroblastoma. From some tumours, e.g. leukaemia, embryonal carcinoma and neuroblastoma, chromosomally relatively stable cell lines have been established found intrumental for such studies. Taken together these studies show (a) that the block in differentiation of malignant cells sometimes can be overcome by non-physiological- (e.g. phorbol ester, dimethyl sulphoxide (DMSO) ) as well as physiological signals (e.g. vitamins and differentiation factors), and (b) that the induced differentiation often, but not always, is similar to that of the corresponding normal cells, i.e. the gene programmes for proliferation/differentiation are orderly executed. The latter fact has made these malignant cell systems acceptable models for studies of various aspects of normal growth and differentiation, e.g. growth- and differentiation factors and their intereaction with the corresponding cell surface receptors, second messenger systems and differentiation-associated gene expression.

The format of the present seminar, the content of which will be briefly summarized in the following, was such that a few overview presentations introduced different systems for studies of growth and differentiation. These were then followed by several talks dealing with some other types of differentiation models, with differentiation associated gene expression and with the possible clinical use of some of the products of such genes.

E. Lehtonen (Helsinki, Finland) described the potential of the mouse embryonal carcinoma (EC) system for studies of multipotential stem cells, i.e. cells with the capacity to differentiate into several distinct cell lineages. He concluded that the EC cells represent primitive ectoderm cells and that the differentiation of such cells in vitro and in vivo is comparable to that of the corresponding normal embryonal cells. The induced differentiation of the EC cells is furthermore orderly and the derived mature cells are non-tumorigenic and mortal. The system has provided extensive information as to the differentiation-associated expression of several cell surface and cytoskeletal proteins and protooncogenes.

K. Nilsson (Uppsala, Sweden) reviewed the various human leukaemia/lymphoma cell line models (e.g. K-562, HL-60 and U-937) and presented evidence that terminal differentiation indeed can be induced in such cell types not only by phorbol esters, DMSO and sodium butyrate (SB) but also by vitamin D3, retinoic acid and several purified haematopoietic growth and differentiation factors at physiological concentrations. The induced differentiation is associated with an arrest in G1, which is irreversible. Several protooncogenes are regulated to increase or decrease their expression during the induction of differentiation of the HL-60 and U-937 cell lines. By transfection studies, using a v-myc gene and a retroviral vector, it has been demonstrated that terminal differentiation may occur only when the myc oncogene is down-regulated, suggesting that the dysregulated, constitutive expression of this oncogene in Burkitt's lymphoma will result in inability of the cells to undergo normal terminal differentiation and concomitant growth arrest.

R. Alitalo (Helsinki, Finland) described that the erythroleukaemia cell line K-562 represents a model for studies of haematopoietic differentiation and that either megakaryoblastic or erythroid differentiation may be induced depending on the inducing agent. The differential expression of the genes for PDGF A and B-chain and TGF-$\beta$ with the two inducible cell lineages was demonstrated by Northern blot analyses of mRNA. The studies thus exemplify cellular network interaction in that the distinct signals may direct the differentiation and that the differentiating cells turn on genes for growth factors that can be utilized either by the maturing cells (autocrine loop) or represent signals for interaction with neighbouring cells (paracrine loops).

C.Harris (Bethesda, Maryland, U.S.A.), in his overview on molecular and cellular

aspects of human lung carcinogenesis, pointed out that development of lung cancer is a multistep process involving aberrations in the pathways of growth and differentiation of bronchial epithelial and pleural mesothelial cells. Lung cancer cells appear to have a selective clonal expansion advantage by being less sensitive to factors inducing terminal differentiation (e.g. TGF- , phorbol ester) and by producing growth factors acting to promote their growth by autocrine loops. The experimental studies with bronchial normal and carcinoma cells also suggest that some of the protooncogenes (e.g. Ha-ras, c-myc and c-raf), like in haematopoietic tumours, after activation play a pathogenetic role in lung carcinogenesis by dysregulating the gene programmes for growth and differentiation. This leads to immortality and the subsequent development of other aspects of the malignant phenotype (the latter usually the result of induced chromosomal instability and the following chromosomal changes).

M. A. Versnel (Rotterdam, The Netherlands) reported on the possible role of c-sis in the pathogenesis of another type of lung cancer - the mesodermally derived malignant mesothelioma. Seven malignant mesothelioma cell lines, but not normal mesothelial cells, all had chromosomal aberrations and expression of the c-sis gene as examined by northern blot analyses. It was furthermore pointed out that examination of c-sis mRNA by in situ hybridisation may be of use for classification of lung and pleural tumours.

L. Wasserman (Tel-Aviv, Israel) concluded from his studies of differentiating MCF-7 breast cancer cells, using DMSO and SB as inducers and some enzymes ( $\gamma$-glutamyl-transpeptidase and alkaline phosphatase) and estradiol binding as markers for differentiation, that there was no ordered but rather a selective pattern of differentiation depending on the inducer used.

M. Ponzoni (Genoa, Italy) described yet another cell system for differentiation studies - neuroblastoma. A new human cell line (GI-ME-N) and its response to Ara-C was examined. The GI-ME-N line was found to be inducible to differentiation using morphology, and expression of neurofilament and neuroblastoma specific antigens as markers. As in the leukaemia/lymphoma cell lines a concomitant inhibition of growth is apparent, suggesting that the induced differentiation was terminal.

The possible association between sensitivity to muscarinic receptor agonists (the effect being measured as $Ca^{++}$ mobilization) and an activated ras oncogene was suggested by J. Heikkilä, Åbo, Finland) from his studies on two human neuroblastoma cell lines containing an activated ras and an amplified myc oncogene, respectively.

The role of oncogenes and the importance of growth and differentiation factors acting via paracrine and autocrine loops were discussed also by P. M. Comoglio (Torino, Italy) in relation to his report on the diagnostic use of an anti-P-Tyr antibody. The antibody, which recognized proteins of different molecular weights in various malignancies, appears to be useful as a tumour marker as P-Tyr was detectable in tumour cells only. The P-Tyr protein of 150 kD on cells of a gastric carcinoma cell line was investigated in some detail and found to be a surface protein. The possible differentiation association of the antigen was, however, not known yet.

M. R. Price (Nottingham, U.K.) reported on the use of a known differentiation associated antigen as a serum tumour marker. He used the NCRC-11 antibody, recognizing high molecular weight antigens expressed on normal secretory glandular epithelia and on epithelial malignant cells. A "sandwich" radioimmunoassay was used to assay the NCR-11 antigen (which may be released from cells in soluble form) into the serum of breast cancer patients and healthy control females. The antigen was detected only in serum of breast cancer patients (41%).

## TUMOUR PROMOTION AND TUMOUR PROGRESSION

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This seminar was concerned with changes which occur in tumour cells or in the host during tumour promotion and progression towards higher malignancy. The interaction of driving forces of tumour diversification on one hand and of host selection on the other seems to generate with time tumour subpopulations or variants with changed genotypic and phenotypic characteristics which have certain growth advantage, become immunoresistant, invasive and eventually metastaic. Many reports dealt with genetic changes such as aneuploidy, mutation rate, gene amplification and role of oncogenes, others also investigated cell surface changes, adhesion molecules, glycosylation changes and changes in expression of major histocompatibility antigens. Two presentations dealt with immune escape from

T cell recognition and immune suppression of NK cells during leukaemogenesis.

V. Shirmacher (Heidelberg, F.R.G.) reported on spontaneous variants derived from a murine T cell lymphoma which greatly differ in vivo in overall malignancy (type of metastatic lesions, organotropism, hind leg paralysis, mortality). Such differences could be associated with cytogenetic changes, changes in cellular adhesiveness and with altered glycosylation of common leucocyte antigens T 200. Plant lectins and monoclonal antibodies were both useful tools to unravel subtle molecular changes of glycoproteins at the cell surface which might hve important impacts on intercellular and cell-substrate interactions.

V. P. Lehto (Finland) gave a detailed report on structure-function relationships of the ras oncogene product p21. The hypothesis that p21 functions like the signal transmitter G proteins was tested using site directed mutagenesis derived p21 mutants to map GTP binding sites, GTPase activity and cell transformation capacity. With this approach a novel TGP binding region could be mapped in the C-terminus of p21. A putative phosphorylation site (TYR 64) was identified and it was proposed to be important in obtaining the transforming conformation of the molecule.

A possible role of ras oncogenes in metastasis was studied and reported by M. Bar-Eli (Beer Sheva, Israel). The major oncogene which showed differential expression in murine T10 fibrosarcoma lines of different metastatic capacity was the Ki-ras protooncogene. Surprisingly, the highly metastatic clones showed a lower expression of specific Ki-ras mRNA and Ki-ras p21 protein than the non-metastatic lines. Transfection of non-metastatic cells with a cloned H-2D$^k$ gene resulted in H-2D$^k$ protein expression, shifting of the cells to high metastatic phenotype and reduction of expression of c-Ki-ras oncogene. An inverse relationship thus seemed to exist within this tumour model between Ki-ras oncogene expression on one hand, and MHC class I antigen expression and metastatic competence on the other. It will be interesting in the future to see (1) how this may be explained at the molecular level, (2) what role the host immune system, in particular the T cell system, may play (immunosuppression via D end class I gene products?) and (3) whether similar assocations may be found in other tumour systems.

Another report from Beer Sheva, Israel, was given by B. Rager-Zisman. It presented evidence for tumour cell mediated immunosuppression at the level of NK cell activity during MoLV (Moloney murine leukaemia virus) induced leukaemogenesis. It was postulated that the progression of virus transformed T cells to a fully developed tumour depends on the ability of these cells to down-regulate NK cell activity and thus evade surveillance.

A. R. Kinsella (Manchester, U.K.) reported on the influence of TPA on the recovery of drug resistant colonies of mouse and hamster cells. TPA was shown to enhance manifold the recovery of mouse cell clones resistant to all three drugs. However, TPA induced enhancement of methotrexate resistant colony recovery was not due to gene amplification in this system.

A very interesting study with clinical tissue specimens was reported by B.Moberger (Stockholm, Sweden). DNA measurements were made on smear preparations and histological sections by direct microspectrophotometry and by flow cytometric determinations of tumour cell suspensions. Specimens were derived from normal and hyperblastic endometrium and of endometrial carcinomas of various stages and histological grade. The investigations gave conclusive evidence that the degree of anaupoloidy of the endometrial carcinomas was the most significant marker for prognosis.

A report by P. E. Schwarze (Oslo, Norway) was concerned with carcinogen-induced hepatocarcinogenesis, in particular with the inhibition of polyploidization of hepatocytes following treatment with 2-acetylaminofluorene (2-AAF). Hepatocytes seemed to be constitutively blocked in their ability to polyploidize and cancers isolated from the host liver consisted predominantly of diploid cells.

Another type of genetic change during multistage carcinogeneis was reported by M. Guminska (Cracow, Poland). Among 12 human urothelial cell lines of different transformation grades (TGr) in vitro (I-III) (from the Fibiger Institute, Copenhagen, Denmark) a nuclear pyruvate kinase (PK) isoenzyme variant was found in the tumorigenic TGr III cell lines only. This isoenzyme variant was inhibited by L-cysteine. A low tumorigenic revertant showed a simultaneous reduction of the sensitivity of PK to L-cysteine inhibition.

## MOLECULAR BASIS AND TARGETING OF CANCER CHEMOTHERAPY

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Progress in cancer chemotherapy has proceeded at a slow pace during the last decade. The screening of a large number of chemicals for cancerostatic action has led to the detection of many compounds that are active against murine tumours, but has yielded little in terms of clinically useful new drugs. In fact, since the discovery of cis-platinum no major breakthrough has emerged. The elucidation of the molecular mechanism of action of anticancer drugs is one of the cornerstones on which the development of improved agents must be based.

A major shortcoming of our current anticancer drugs is that they are all unspecific. Almost all of them target DNA synthesis or the mitotic process, and hence they act also on rapidly dividing normal cells with consequent serious dose-limiting side effects. An important new approach to achieve specificity is to utilize monoclonal antibodies (MoAbs) to target drugs to the tumour cells. MoAbs directed against tumour associated surface antigens have been conjugated with conventional cytotoxic drugs, radionuclides or protein toxins.

The mechanism of action of immunotoxins (ITs) was discussed by A. Pihl (Oslo, Norway). The ITs are conjugates of MoAbs with highly active plant or bacterial toxins, such as ricin and diphtheria toxin, or their active subunits. Since the toxins used as components of ITs kill cells by inhibiting cellular protein synthesis, hopefully the ITs may be active also against slowly growing tumours which constitute the main problem in chemotherapy of cancer.

In recent years a large number of ITs have been prepared. They have turned out to have widely different toxicities and specificities for reasons that are not immediately obvious.

In their native form the toxins used for preparation of ITs consist of two polypeptide chains with different functions, a B-chain that binds the toxins to receptors present on the surface of eukaryotic cells, and an A-chain which is responsible for the toxicity. Since the B-chains will bind to all eukaryotic cells, most investigators have used only the A-chain for preparation of ITs to achieve the desired specificity.

The molecular mechanism of action of the toxins and the corresponding ITs is now known in considerable detail. The first obligatory step is binding to receptors on the cell surface. The ITs, like the native toxins, are then internalized by receptor mediated endocytosis. The active agents, the A-chains are then translocated from vesicular compartments into the cytosol where they inhibit cellular protein synthesis. Different toxins, and the corresponding ITs, may enter the cytosol from different compartments. Diphtheria toxin enters from acid vesicles, whereas ricin seems to enter from the neutral trans-Golgi compartment.

The extreme potency of ricin and related toxins is due to the fact that the A-chains are enzymes, and there is evidence that the entry of a single A-chain into the cytosol is sufficient to kill a cell. The A-chains inhibit protein synthesis either by inactivating elongation factor 2 by ADP-ribosylation (diphtheria toxin, pseudomonas exotoxin A), or by inactivating the 60S subunit (ricin, abrin, modeccin, viscumin, volkensin). Until recently the mechanism whereby this occurs has been obscure. Now Japanese workers have shown that the plant A-chains are glycosidases with extreme specificity. They act by splitting off an adenine residue from a specific nucleotide near the 3' end of the 28S rRNA.

The activity and specificity of the immunotoxins depend on a number of factors such as the uniqueness, surface density, and intracellular routing of the antigens targeted, the specificity and affinity of the antibodies, the nature of the toxins, the chemical nature of the linkage, as well as metabolic properties of the cells. In general, A-chain ITs are less toxic than ITs prepared from holotoxins since the toxin B chains somehow facilitate the translocation of the A-chain though the vesicular membranes. The mechanism whereby this occurs is complex and still inadequately understood. The question whether the B-chain gal-binding site is involved in this facilitation is still controversial. The use of holotoxins as components of ITs will give unspecific toxicity unless the IT is prepared in such a way that the B-chain binding sites are unexposed, or they are removed or modified. In experiments in vitro, unspecific action can be avoided by incubation in presence of lactose. The sensitivity of cells to ITs containing holotoxins is influenced by inherent metabolic properties of the cells. The widely different sensitivities of different melanoma cell lines to an abrin-IT are correlated with their sensitivity to native abrin which seems to reflect different abilities of the cell lines to translocate the A-chains of the toxins and

the ITs into the cytosol.

So far ITs have mainly been used in vitro to purge patients bone marrow of malignant cells or donor marrow of cytotoxic T cells involved in graft versus host disease. Although the in vivo use poses a number of additional problems, in vivo trials in human cancers are now under way.

One of the important cancer forms where immunotoxin therapy is now being attempted is gastro-intestinal carcinoma where carcinoembryonic antigen (CEA) may be used as target. Since immunotoxins are taken up by receptor mediated endocytosis, factors potentiating endocytosis might possibly enhance the cytotoxic action of immunotoxin. This possibility was studied by N. Berry and collaborators (Nottingham, U.K.) who reported that the endocytosis of an anti-CEA antibody by a gastric carcinoma cell line, MKN-45, could be enhanced by other antibodies defining epitopes common to CEA and normal cross reacting antigen (NCA).

Anti-tumour antibodies labelled with radioactive isotopes represent a promising new approach to targeted radiotherapy. Hopefully, by the use of isotopes with suitable physical properties (type of radiation, energy, half life) the destruction of metastases may be obtained. B. Fermor (London, U.K) reported that two [131]-I-labelled monoclonal antibodies, C215 and C242, were able to inhbibit the growth of transplanted murine mammary tumours.

An important step in the use of MoAbs for targeting of drugs is to demonstrate that the antibodies localize specifically to tumour tissue. P. Vihko (Oulu, Finland) demonstrated that high-affinity MoAbs, specific for human prostatic acid phosphatase (PAP), could be used to localize metastases from prostatic cancer by radioimaging. The antibodies exhibited strict specificity for PAP which has an antigenically unique region not present in acid phosphastases from other sources.

In five patients who were candidates for radical prostatectomy, $F(ab')_2$ -fragments labelled with Tc-99m or In-111 were injected bilaterally into the periprostatic space. In one of the patients, lymph node metastases were visualized. Subsequent pelvic lymphadenectomy confirmed that the lesions incorporating radioactivity were indeed metastases. The data indicate that these antibodies can be used for preoperative staging of prostatic cancer.

A novel approach to cell-specific killing was discussed by L. M. Glode (Denver, U.S.A.). A toxin gene can be placed under the control of other DNA regulatory elements and thus be expressed only in cells which contain trans-activating factors unique to their phenotype. Subsequent induction of transcription and translation of the toxin gene should then lead to cell death. It was suggested that malignant cells which express marker proteins or other characteristics via trans-activators not found in normal cells might be eliminated by controlled toxin gene expression and that this new approach may allow targeting of unique intracellular phenomena such as the expression of a non surface antigen protein.
The strategy outlined for inducing cancer cell suicide involved transfection into human cells of a diphtheria toxin A-chain gene, the expression of which is regulated by a heat shock (HSP 70) promoter and transient exposure of the cells to 42° C.

Since the wild-type diphtheria toxin A-chain might prove too toxic in the event of leakiness, the coding sequence for an attenuated diphtheria toxin A-chain, tox 176, was cloned and vectors which included the regulatory elements of the heat shock response gene were constructed which either integrated into cell DNA or remained episomal. Experiments to induce cell killing were in progress.

Considerable difficulties must be overcome before this interesting new approach can be successfully used in the treatment of human cancers since success of such therapy will depend on the development of efficient means of delivering exogenous genes to tumour cells. It was suggested that defective recombinant viruses may be used for delivery of toxin genes to accessible populations of cancer cells, e.g. in bone marrow, skin or bladder cancers.

Different aspects of the mechanism of action of fluoropyrimidines were discussed by two Swedish groups. U. and S. Lonn (Stockholm, Sweden) reported that 5-fluoropyrimidines induce DNA lesions by two different mechanisms. When cells from a human adenocarcinoma cell line were prelabelled with DNA and treated with 5-FU, fragmentation of DNA was detected after lysis of the cells in dilute alkali. The fragmentation was visualized by agarose gel electrophoresis. Labelled 5-FU was incorporated into the DNA. In cells without active DNA synthesis (cells treated with aphidicolin) a second type of DNA lesions not involving incorporation of the drug, was seen. This mechanism probably involves inefficient repair of normally occurring DNA lesions. The inefficiency of the DNA repair could be due to the known 5-Fu-induced inhibition of nucleotide synthesis.

U. Stenram (Lund, Sweden) reported data supporting the view that adequate protein and amino acid nutrition may be important during 5-Fu therapy. To mimic the clinical situation with liver metastases, an experimental adenocarcinoma of the colon was transplanted into the liver of rats. In protein deprived rats the incorporation of 5-Fu, infused via the hepatic artery, into liver and intestinal RNA, but not into tumour, increased significantly. The increased incorporation into normal tissues was largely eliminated by parenteral feeding of amino acids.

The use of $\alpha$-difluoromethylornithine (DFMO), an irreversible inhibitor of ornithine decarboxylase, as a potentiator of chemotherapy was considerd in two papers. DFMO induces depletion of cellular polyamines and exerts cytostatic activity in different in vivo and in vitro experimental tumour system. T. Kremmer (Budapest, Hungary) reported that when mice with i.p. P388 leukaemia were given DFMO continuously in the drinking water, a marked lowering of intracellular putrescine concentration in the leukaemic cells was seen. Partial depletion of intracellular polyamines, induced by DFMO, enhanced the cytotoxicity of cyclophosphamide in P388 leukaemia and increased markedly the lifespan of tumour bearing animals above that of animals given the alkylating agent alone, and a significant number of long time survivors was obtained.

The mechanism of resistance to DFMO was discussed by F. L. Meyskins (Tucson, U.S.A.). Three human melanoma cell lines that were resistant to DFMO were studied. In a clonogenic assay, all three cell lines were able to overcome growth arrest by utilization of N-acetylputrescine, an excretory product of polyamine metabolism. It was suggested that DFMO therapy in human melanoma might be potentiated by the use of an inhibitor of N-acetylputrescine deacetylase.

A possible antitumour effect of stearic acid was reported by B. Fernor (London, U.K.). Since decreased membrane rigidity is one of the characteristics of malignant cells, resulting in part from the desaturation of stearic acid into oleic acid, the authors investigated the influence of stearic acid on the colony-forming ability of two human tumour cell lines and on tumour development in vivo. The preliminary data reported suggested that stearic acid, but not oleic acid, kill human cells in vitro. Also, it was reported that s.c. injection of stearic acid prevented tumour development in a significant proportion of rats with nitrosomethyl urea-induced mammary carcinoma.

Failure of chemotherapy of cancer is frequently due to the presence of resistant tumour cell populations. Studies of the mechanisms of resistance to drugs are therefore of great interest. J. R. Warr (York, U.K.) reported a novel form of vincristine resistance. Frequently, vincristine-resistant cell lines exhibit multi-drug resistance which often is associated with reduced drug accumulation. The multi-drug resistance phenotype can be reversed by the presence of verapamil and several other calcium channel blocking or membrane active agents. Usually such cells have very high levels of a 170 kD membrane glycoprotein - the P-glycoprotein. Warr had observed two vincristine-resistant CHO lines which showed considerable hypersensitivity to verapamil in the absence of vincristine. The cell lines were also sensitive to other membrane acting agents which are not calcium channel blockers, and the rate of calcium accumulation in the absence and presence of verapamil was similar in the vincristine resistant cell lines and the controls. Thus, in these cell lines, the vincristine resistance did not seem to involve calcium channels. Possibly, analysis of the membranes of these cell lines might provide clues as to the nature of the P-glycoprotein function and multi-drug resistance.

A. M. Kroon (Groningen, Netherlands) reviewed the extensive work of his group on mitochondria as intracellular targets for anticancer therapy. The mitochondria are indispensable for aerobic metabolism in normal as well as cancer cells. Cancer cells in general contain fewer mitochondria than normal cells and turn over more rapidly. Therefore, agents which specifically inhibit mitochondrial protein synthesis, such as the tetracyclins, might be expected to deplete the functional respiratory chain in cancer cells with decreased energy-generation capacity and, eventually, cell proliferation arrest. This has been shown to occur in a variety of experimental tumour systems. The mitochondrial system may be involved also in some of the effects of other cytostatic agents such as doxorubicin which interferes with mitochondrial membrane function, methotrexate which inhibits mitochondrial inner membrane development, and cytarabin (Ara-C). Possible applications of tetracyclin treatment in human cancer include: (1) treatment preceding surgery and/or radiation, (2) combination therapy

with other cytostatic agents, given simultaneously or intermittantly and (3) as the only drug in cases for which no alternative treatment is available.

The availability of suitable test systems is essential for the development of improved therapeutic regimens. Most previous models are based on rodents and their relevance to the human situation may be questionable. C. Mothersill (Dublin, Ireland) reported her experience with a model involving explants from human tumour and surrounding normal tissues in the same patient. The amount of outgrowth of cells from a small piece of tissue over a 2 to 3 weeks period was measured. The system permits assessment of the effect of chemotherapy and radiation, singly and in combination, and optimal conditions can be worked out. Data on both oesophageal adeno- and squamous cell carcinomas and bladder carcinomas indicated that by manipulating the radiation and cytotoxic doses a selective killing of tumour cells can indeed be achieved.

## MONITORING OF CARCINOGEN EXPOSURE

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This seminar contained presentations from the monitoring of exposure to detection of effects. Antibodies have been raised against aflatoxin B1 and they have been used to screen urines of a Danish population (L. O. Dragsted and H. Antrup - Copenhagen, Denmark). The urines showed antigenicity and dietary studies pointed to higher excretion of aflatoxin-like substances in response to diets containing dairy and meat products. Structural characterization of the substances is under way. C. C. Harris (Bethesda, U.S.A.) reported on the presence of antibodies in some humans against benzo(a)pyrene tetrols after occupational exposure to polycyclic aromatic hydrocarbon.

R. J. Laib (Dortmund, F.R.G.) surveyed data on the DNA binding in vitro and in vivo of vinyl chloride and vinyl bromide. The results imply the role of an epoxide intermediate in the formation of 7-(2-oxoethyl)guanine in DNA. Binding of butadiene to DNA was studied in rats and in mice. One adduct was identified as 7-(1-hydroxy-3-buten-2-yl)guanine produced by a metabolically formed mono-epoxide.

Studies on the human monitoring of occupationally exposed population were reported by three speakers, C. C. Harris, K. Vahakangas (Oulu, Finland) and K. Hemminki (Helsinki, Finland). The methods used were immunological assays for benzo(a)pyrene modified DNA. [32]-P-postlabelling technique and synchronous fluorescence spectrophotometry. In foundry workers exposure levels to polycyclic aromatic hydrocarbons were shown to correlate with the presence of polycyclic adducts in white blood cell DNA.

C. C. Harris discussed possibilities to use metabolic differences in humans as tests for susceptibility to cancer. In particular, acetylation rates and debrisoquine metabolism have been used in this regard. Harris' own data suggest that more extensive metabolizers of debrisoquine are among lung cancer patients with squamous cell carcinoma but not those with adenocarcinoma.

Further tests on inherent predisposition to cancer have been revealed by molecular genetics using analysis for size polymorphism or insertion/deletion polymorphism. Data seem to point to a higher prevalence of rate alleles of H-ras gene in lung cancer. Furthermore, frequent reduction to homozygosity of several genes is seen in such patients.

Toxicity and genotoxicity testing have carried out with betel extracts using cultures of human buccal cells, as reported by K. Sundqvist (Stockholm, Sweden). Betal extract caused genotoxicity in buccal cells and 3-(methylnitrosamine)propionaldehyde proved to be a potent compound present in the extract.

S. Simi (Pisa, Italy) showed that methotrexate induced certain patterns of chromosomal breakage in Chinese hamster cell lines. S. Parodi (Genoa, Italy) presented an extensive analysis of the quantitative results from various short-term tests in the prediction of cancer risk to experimental animals.

## SUSCEPTIBILITY TO CANCER

Reported by: **Herman Autrup**

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One of the puzzling questions in human carcinogenesis is, why does a clinically evident cancer not develop in all individuals that have been exposed to the same amount of carcinogenic agent, e.g. cigarette smokers in case of bronchial carcinoma? Are some people more susceptible than others due to heritable factors or do

other factors also play a role? Host determinants in human carcinogenesis include exposure to the carcinogenic agents, exposure to other compounds that modify the oncogenic process, e.g. limit the availability of carcinogenic material, and predisposing genetic factors.

Most environmental carcinogens require metabolic activation to exert their carcinogenic activity. The ability to activiate/deactivate a chemical carcinogen has been suggested to be a host determinant in carcinogenesis. The genes coding for the enzymes responsible for the metabolic activation of carcinogens and for enzymes responsible for the repair of the DNA damage induced by the carcinogen have been termed carcinogenesis susceptibility genes.

O. Pelkonen (Oulu, Finland) presented data on cytochrome P-450IA1 and one of its associated enzyme activities, arylhydrocarbon hydroxylase (AHH) in human tissues. The inducibility of AHH in mouse is associated with susceptibility to polycyclic aromatic hydrocarbon (PAH) carcinogenesis, and the inducibility of AHH in cultured human lymphocytes has previously been associated with increased risk for developing bronchiogenic carcinoma, but in a recent study no difference in inducibility of AHH between the lung cancer and the control group could be detected.

Using a monoclonal antibody against rat cytochrome P-450IA1 (Mab 1-7-1), the enzyme was shown to be present in human liver and placenta tissues. However, the antibody inhibited AHH 80% in the placenta, but only 30% in adult and 10% in fetal liver. The results suggest that the placenta mainly contains the form of P-450 that is known to be induced by tobacco smoke.

In addition, Mab 1-7-1 inhibited the formation of benzo(a)pyrenediolepoxide-DNA (BPDE-DNA) adducts in placenta but not in liver, when microsomes from these tissues were incubated in the presence of benzo(a)pyrene and DNA. The amount of BPDE-DNA was quantitated by synchronous fluorescence spectrophotometry. In human lung cells an association between the amount of BPDE-DNA and the concentration of BP in the incubation mixture suggests that the measurement of BPDE-DNA may be a good and accurate measure for the exposure to BP. The adduct was detected in 14/17 coke oven workers.

Recently the mouse P-450IA1 gene has been cloned. In mouse liver a good correlation between the amount of mRNA of P-450IA1 and the AHH activity was established, suggesting that this molecular biology approach may offer reliable and more accurate approach to identify individuals with increased risk of developing chemically induced cancers.

Smoking of cigarettes is considered the major etiological factor in the induction of lung cancer. The chemical nature of cigarette smoke is quite complex, and in addition to the carcinogenic compounds, the smoke contains several factors that may modify the carcinogenic process. R. Grafström (Stockholm, Sweden) reported on some of the biological effects of tobacco smoke condensate (TSC) on human bronchial epithelial cells cultured at serum free conditions. Cell survival and growth rate were determined by the clonal growth assay and the DNA damaging activity by the alkaline elution technique. The effects were concentration and time dependent. The TSC of regular cigarette did also deplete cellular thiols, compounds that are thought to protect cells against genotoxic agents, e.g. the ones found in TSC. The semi-volatile fraction of TSC was most active to deplete thiols but was low in genotoxic activity. TSC and its different fractions did also induce terminal differentiation. The biochemical effects of TSC in human bronchial cells could be eliminated or minimized by addition of N-acetyl-cysteine to the culture medium suggesting that thiols play an important role in the protection against TSC induced cellular damage.

Selenium is another important compound that influences the carcinogenic process, as people living in areas with low selenium content of the soil has an increased risk of developing cancer. Se is part of the gluthathione peroxidase enzyme that is important in the oxidoreductase balance. M. Mikac-Devic (Zagreb, Yugoslavia) reported on an extensive survey of the selenium levels in Yugoslavian subjects. The mean level of sera of normal individuals was 64.2 µg/L and a mean urine level of 6.94. The latter compares with 124.4 in an average Canadian population. No difference between age, sex, and smoking was observed. The low level in the Yugoslavian population was associated with low dietary intake of Se, e.g. cereals contain only 0.01 µg/kg compared with 1.5 µg/kg in the U.S.A. In a case control study colon cancer patients were compared to a control group but no difference in serum or urine level of Se was observed. It was recommended to take preventative steps by increasing the Se level in the soil by adding Se compounds to the fertilizers as level of Se in cereal is a function of soil level.

Bile acids and small bowel resection have been recognised to promote carcinogenesis in the colon of experimental animals. G.

Appleton (Bristol, U.K.) reported that supplementation of the drinking water with calcium lactate (24 g/1) inhibited both the growth stimulation and the tumour frequence. Male rats were treated with a carcinogenic dose of azoxymethane. This treatment was followed either by 80% resection of the small bowel or transection with reanastomosis. Seven weeks after the surgical procedure some of the animals in each group were treated i.p. with vincristine and the metaphases counted in the mid-descending colon. The resection itself increased the crypt cell production rate (CCPR) but supplementary calcium inhibited both this increase as well as it significantly inhibited the CCPR in rats that were transected. In the carcinogensis experiment the tumour yield was nearly halved in the group receiving calcium.

During the discussion it was emphasized that consumption of low fat milk protected against colon cancer by the formation of insoluble calcium salts of bile acids, and thereby removing potential promoting agents. This could also be supported by the observation made in a comparative epidemiological study that the consumption of milk products was the only difference between high and low risk groups.

Patients with familial polyposis and other hyper-proliferative disorder have an increased risk of developing colon cancer. In normal colon the proliferative activity is limited to the lower portion of the crypts and is almost absent at the surface. Using an autoradiographic technique M. Ponz de Leon (Modena, Italy) reported that there wa no difference in number of labelled cells in the different anatomical segments of normal colon. There was no difference in number of labelled cells in the lower compartments between control and in colon of patients with cancer or polyps. However, an increase in number of labelled cells in the upper third of the crypt was found in cancer patients. An increase in number of labelled cells was also noted in the mid-portion of the crypt in patients with polyps or cancer. The results suggest that these disorders are associated with an expansion of the proliferative compartment and that the presence of proliferative cells in the upper layer could be used to discriminate between subjects with or without intestinal cancer.

An alternative to prevention is early diagnosis, and T. Cotter (Kildare, Ireland) described the production and characterisation of a monoclonal antibody that reacted with blood and bone marrow cells from leukaemia patients whereas only 7 out of 130 normal individuals showed a positive response in a blood smear. The antibody was produced against HL-60 cells. The antigen had a molecular weight of 50 Kd, and was not sensitive to either trypsin, papain, or neuraminidase. Using the alkaline phosphatase monoclonal anti-alkaline phosphatase method, it was shown that the location of the antigen was cytosolic.

In bone marrow cells, using an individual immunofluoresence technique, 10% of normal cells reacted whereas it was higher in leukaemia patients but no correlation existed between the type of leukaemia and number of positive cells.

No association between major histocompatibility complex and cancer risk has been established but H. -J. Keyserlink (Berlin, F.R.G.) presented data from a multicentre analysis on HLA antigens and testicular cancer. In patients with pure seminoma, a statistical significant increase in HLA B was found compared to control. The data were only preliminary but suggested that the susceptibilty or resistance to develop seminoma is HLA associated but that more studies are needed to establish a clear association.

## EPIDEMIOLOGICAL APPROACHES TO CARCINOGENESIS

Reported by: **Matti Hakama**

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The chairman of the seminar, D. Zaridze from U.S.S.R., was unable to attend. In his absence the background presentation on diet and cancer was given by M. Hakama from Finland. He also described the large scale serum sample bank in Finland which was linked with the Finnish Cancer Registry. He concluded that the estimate of one third of cancers being caused by dietary factors is within the reasonable limits. J. Weisburger from U.S.A. was kind enough to supplement the symposium by an overview on the epidemiologic data on nutrition and cancer by presenting the views and results of the American Health Foundation.

H. Adlercreutz from Finland presented results on the case control study of hormones and hormone-like compounds on risk factors of breast cancer and their correlation with diet. H. Adlercreutz concluded that the western type diet is associated with high testosterone, estrone and estradiol levels and low sex hormone binding globulin binding capacity and, thus, is likely to increase the risk of breast cancer and other homrone dependent cancers.

D. Rose from U.S.A. compared the epidemiology of breast cancer in U.S.A. and Finland. Both populations have high dietary fat intake but the risk of breast cancer is much higher in the U.S.A. than in Finland. Therefore other risk factors were studied and it appeared that there were only small dfferences also in the reproductive risk factors and also they appear insufficient to explain the higher breast cancer risk in the U.S.A.

M. Ponz de Leon described the colorectal tumour registry in Modena, Italy. The genealogical tree was carefully traced and it was found a relative risk of 2.5 for parents and 9.0 for siblings. The study suggested that genetic factors may be involved in about 20 percent of colonic tumours.

An overview on the human papilloma virus infections and risk of cervical cancer was presented by K. Syrjänen from Finland. He pointed out the results of both case control studies and cohort studies demonstrating the high correlation between the exposure to selected HPV infections and risk of invasive and preinvasive cervical lesions. The follow-up study of his group revealed aspects in natural history and potential of malignancy of. the HPV infections in a Finnish population.

E. Pukkala described the joint effort of Finnish Cancer Registry and Geological Survey of Finland to produce Finnish Cancer Atlas and to correlate the cancer risk with geochemical data and with other background variables by geography. Such data include trace elements, industrial structure, social welfare, etc.

The audience showed considerable interest in the presentations and all the papers were actively discussed. The most active participants moved to the Finnish Cancer Registry where the discussion on the cancer epidemiology was continued.