— to provide state of the art information about cancer therapy.

The EuroCODE computer network links the EORTC Data Center and other major cancer trials offices. There are 409 users from 28 countries.

Clinicians and investigators throughout Europe can access databases and information provided by these centres, and randomise patients into cancer clinical trials. EuroCODE is connected to certain national networks, but, in addition, any individual investigators can access EuroCODE by using a modem connected to a PC and a telephone line.

The following services are available on EuroCODE:

1. The registration/randomisation of patients into EORTC collaborative studies 24 hours a day, 365 days a year. Eligibility of the patient for the study is automatically checked, and a treatment arm is allocated in case of a randomised study.
2. Access to PDQ, Physician Data Query, a database of cancer information and cancer trials compiled by the US National Cancer Institute (NCI). It contains state of the art information on prognosis, staging, histology and classification of cancer. In addition, it provides an extensive register of physicians, cancer institutes and working groups.
3. In addition to PDQ, a list of ongoing EORTC studies is available on EuroCODE. A detailed register of U.K. trials is currently being compiled by the UKCCCR, and will also be accessible over EuroCODE. This register will be extended with trials from other European countries.
4. Each EuroCODE user has an electronic mailbox. Electronic mail (Email) allows the users to send or receive mail to or from the EuroCODE centres, or exchange mail with other EuroCODE users. EuroCODE provides a list of all EuroCODE users, including a search facility. This allows users to send electronic mail to other users.
5. A schedule of meetings of EORTC cooperative groups and other important associations is provided.

CONCLUSIONS

The EORTC Data Center provides a wide range of expertise to investigators in the conduct and coordination of phase II and phase III cancer clinical trials. In addition to classical aspects dealing with trial design and analysis, speciality units have been developed dealing with phase II trials, leukaemia studies, meta-analyses, quality of life and health economics. In this way the Data Center can better serve the needs of the clinical cooperative groups, and increase the quality and efficiency of the services it provides.

Through the conduct of large scale multinational trials, EORTC studies provide convincing results which may be used to rapidly influence clinical practice in oncology, not only in Europe, but also on a worldwide basis, thereby improving cancer treatment and decreasing the cancer mortality rate. More generally, participation in large multinational studies, such as that conducted by the EORTC, have a broad impact on clinical practice due to their important educational role with respect to all aspects of cancer treatment. This is reflected by the fact that numerous studies have suggested that patients treated in clinical trials have a better survival than patients not included in trials.

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Apoptosis

Fifth Pezcoller Foundation Symposium, Trento, Italy, June 9–11, 1993

Enrico Mihich and Robert T. Schimke

AVANT GARDE annual symposia on the molecular biology of the cancer cell and related therapeutic implications have been supported by the Pezcoller Foundation since 1989. These are held on alternative years in Trento and Rovereto: free discussions among participants are emphasised which are conducive to the formulation of new working hypotheses and collaborations. Thus, through these scholarly events, the Foundation has contributed significantly to scientific progress in oncology. The first four symposia focused on *Drug Resistance: Mechanisms and Reversal* (1989); *Therapeutic Implications of the Molecular Biology of Breast Cancer* (1990); *Tumour Suppressor Genes* (1991); and *Adhesion Molecules: Cellular Recognition Mechanisms* (1992), and the proceedings were published by John Libbey CIC, Rome, Italy (1989, 1990); Edigraf S.r.l., Rome, Italy (1991); and Plenum Press, New York, U.S.A. (1992).

The fifth symposium was dedicated to *Apoptosis* and the mechanisms involved in it. It was held in Trento on June 9–11, 1993 and included 18 invited participants (see Appendix). The complete proceedings of the meeting will be published by Plenum Publishing Corp., New York, U.S.A.

The genetic control of programmed cell death (PCD) in the nematode *C. elegans* was discussed by Dr Horvitz. This system represents a good model for the apoptotic changes occurring in

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vertebrates. One of the 11 genes involved in PCD acts as a developmental "switch" gene and actually protects the cell by controlling a decision point between cell survival and PCD.

Dr Papermaster discussed apoptosis as a mechanism of inherited retinal degeneration and its determinants.

The role of wild type (wt) p53, a tumour suppressor gene, in inducing PCD was discussed by several participants.

Dr Oren was the first to show that the product of wt p53 gene determines apoptosis. This was confirmed in several different tumours, both haematological and non-haematological; wt p53 was also required for the induction of apoptosis by ionising radiation or by adenovirus E1A oncoproteins. A decision point in late G_1 phase was identified from which the cell can proceed towards PCD or not, and which is affected by wt p53.

The regulation of apoptosis by the transforming gene products of adenovirus was discussed by Dr White: E1A induces PCD whereas E1B suppresses it and allows virus reproduction to proceed unimpeded by cell death. The blockage of E1A-induced apoptosis by *bcl-2* appears mediated through a blockage of wt p53. However, the mechanisms by which E1A induces PCD are not yet clear. The information available to date suggests the existence of multiple pathways for the regulation of apoptosis.

Dr Kastan discussed cell cycle checkpoints that are responsive to DNA damage, particularly the wt p53 dependent G_1 checkpoint. Signal transduction pathways are activated following certain types of DNA damage, depend on the normal function of wt p53, and influence the stability of the genome and the effects of cell survival signals. The loss of the wt p53 dependent G_1 checkpoint function leads to increased genetic abnormalities after DNA damage, as seen in a number of human tumours.

A "switch" directing cells towards proliferation or the development of PCD is controlled by c-*myc* protein; *bcl-2* determines the direction of the "switch".

Down regulation of *myc* is required for cell arrest, as discussed by Dr Evan. *Myc* usually stimulates cell proliferation, but if growth is inhibited by other factors, PCD ensues. *Myc*-induced PCD does not seem to be cell cycle phase specific. A scenario was discussed in which *myc* is induced by mitogens or certain growth factors, stimulates growth in the presence of survival factors, and induces PCD in the presence of growth inhibiting factors.

Dr Cleveland discussed the role of apoptosis in the regulation of haematopoiesis. The 32Dc13 murine myeloid cells are interleukin (IL)3-dependent for growth. Upon IL3 withdrawal, c-*myc* is downregulated, the cells are arrested in G_1 phase and from there proceed towards PCD. It was pointed out that *myc* affects a switch point leading to cell cycle progression or apoptosis depending on additional signals. *Myc* product is a transcription factor which appears to regulate, among others, the expression of ornithine decarboxylase (ODC), which is necessary for G_1 progression.

As indicated by Dr Hockenbery, IL3 deprivation-induced PCD in promyelocytic cells can be prevented by *bcl-2*. However, IL2 or IL6 deprivation-induced PCD is independent of *bcl-2*, this supporting further the existence of multiple pathway of apoptosis. In thymic development, *bcl-2* may function as a survival factor in positively selected thymocytes; it is also present in the intestinal crypt cells which are very sensitive to ionising radiation. Whether *bcl-2* acts on the induction of the events leading to apoptosis or on the apoptotic machinery *per se* is not clear.

The generation of an antigen-specific immune repertoire was discussed by Dr Ashwell. Negative selection of double positive ($CD4^+$ $CD8^+$) cells in the thymus occurs by PCD following binding of T cell receptors by ligands; steroids are also known to induce PCD in the thymus. When both ligand binding and steroids are applied, there is mutual antagonism and no PCD ensures: this may be a basis for positive selection of single positive cells. Pregnenolone is synthesised in radioresistant thymic cells. Retinoic acid affects negative selection processes. Steroids and antigen binding to T cell receptors appear to be critical factors in determining PCD-mediated negative and positive selection in the generation of the immune repertoire.

Dr Green discussed the role of c-*myc* and Fas/APO-1 in the PCD of clones of lymphocytes with potential for self reactivity. Based on antisense intervention, it was demonstrated that c-*myc* is required for PCD to occur. The product of this gene affects a switch decision point in conjunction with additional environmental factors: PCD occurs when T cell receptor–ligand complexes inhibit proliferation, and proliferation occurs when PCD is inhibited by *bcl-2* or growth factors. Fas/APO-1, the protein present on thymocytes and activated lymphocytes, determines PCD associated with peripheral T cell selection, as discussed by Dr Green and Dr Krammer.This molecule is a cysteine-rich transmembrane protein which belongs to the tumour necrosis factor (TNF) receptor super family. Ligand binding to it leads to PCD: this may be the main mechanism of peripheral selection/deletion of autoreactive immune effector cells and tolerance inducation.

The role of calcium ions, of intracellular pH changes and of endonucleases function was discussed in relation to the effects of certain anticancer drugs and the regulation exerted by polyamines.

Dr Nicotera discussed the role of intracellular ions and ion signalling in determining apoptosis with emphasis on Ca^{2+}. Intracellular Ca^{2+} signals activate apoptosis, and at least three different Ca^{2+}-dependent mechanisms may be related to the triggering of the nuclear changes occurring in apoptosis: activation of Ca^{2+}-dependent endonucleases causing DNA fragmentation; modification of chromatin conformation resulting in increased susceptibility to nucleases and alterations in gene expression.

The regulation of chromatin conformation by Ca^{2+} was studied in isolated liver nuclei, where chromatin unwinding was favoured by heparin and reversed by spermidine. Following relaxation of chromatin higher order structure consequent to ion changes in the nucleus, and given the appropriate factors, genes involved in apoptosis may be transcribed.

Dr Isaacs discussed the occurrence of PCD in prostate cells after androgen ablation: new proteins are made, cell proliferation stops, polyamines and nuclear histone H1 are decreased, free Ca^{2+} from extracellular pools are increased, genomic DNA conformation changes with increased accessibility of the linker region to endonucleases, DNA breaks ensue. Elevation of Ca^{2+} and consequent activation of PCD in androgen-independent prostate cancer cells are induced by thapsigargin. This indicates the possibility that this sub-population of tumour cells, which is insensitive to growth factor withdrawal, may also be induced to undergo PCD.

Dr Eastman focused on multiplicity of stimuli activating different signal transduction pathways and ultimately leading to endonucleases activation and apoptosis. The importance of phosphorylated membrane ion transporters for cell protection from PCD was emphasised. For example, CHO cells exposed to cisplatinum are arrested in G_2, a process related to a decrease in $P34^{cdc2}$ kinase activity: after p34 dephosphorylation, aberrant

mitosis takes place with unequal chromosome segregation, followed by progression to G_1, detachment from extracellular matrix and apoptotic DNA digestion. The hypothesis was formulated that, in this case, the loss of a signal from the extracellular matrix led to intracellular acidification and DNAse II activation.

The role of transcriptional factors in the regulation of cell growth by type I interferon (IFN) genes (α and β) was discussed by Dr Taniguchi. Interferon regulatory factors 1 (IRF-1) and 2 (IRF-2) are transcriptional regulators, IRF-1 a transcriptional activator, IRF-2 a repressor of IRF-1. It was proposed that the ratio between two IFN transcriptional regulators with opposite function play a role in regulating cell growth; IRF-1 appears to be a tumour suppressor gene; one or both IRF-1 alleles are deleted in human myelodysplastic diseases and leukaemia.

The effects of cycle phase specific drugs on the integration of cell cycle processes and the operation of cycle checkpoints was addressed by Dr Sherwood. In HeLa cells, the apoptotic processes could start at any phase of the cell cycle, indicating that the related biochemical machinery is present throughout the cycle. However, the rate at which apoptosis occurs is influenced by the site on the cycle where the process is being triggered.

In conclusion, the multiplicity of mechanisms determining PCD was extensively discussed. The role of genes, like wt p53 or C-*myc*, and accessory factors in directing cells through "switch" decision points towards proliferation or PCD was demonstrated in various cellular systems. The reduction of PCD, by growth factors or by products of genes, like *bcl*-2, and the induction of PCD by the withdrawal of hormones and growth factors, or by DNA damaging and anticancer drugs, were described. The function of PCD in haematopoiesis and in negative and positive selection during the development of the immune repertoire was discussed. The cascade of events resulting in apoptotic death in each of the models examined and the factors regulating them were extensively discussed: gene transcription, cell cycle control mechanisms, endonuclease function and intracellular ions concentration were emphasised.

APPENDIX

The Program Committee consisted of Drs E. Mihich, Roswell Park Cancer Institute, Buffalo, NY (Chairman); J.M. Bishop, G.W. Hooper Research Foundation, San Francisco, CA; A. Levine, Princeton University, Princeton, NJ; D.M. Livingston, Dana Farber Cancer Institute, Boston, MA; and R.T. Schimke, Stanford University, Stanford, CA.

The 18 invited participants were Drs H. Robert Horvitz, Howard Hughes Medical Inst. Res. Lab., Massachusetts Inst. of Technology, Cambridge, MA; David S. Papermaster, University of Texas Health Science Ctr., San Antonio, TX; Moshe Oren, Weizmann Institute of Science, Rehovot, Israel; Eileen White, Rutgers University, Piscataway, NJ; Gerard I. Evan, Imperial Cancer Research Fund, London, U.K.; John L. Cleveland, St. Jude Children's Research Hospital, Memphis, TN; Pierluigi Nicotera, Karolinska Institute, Stockholm, Sweden; Michael B. Kastan, Johns Hopkins Oncology Center, Baltimore, MD; John T. Isaacs, Johns Hopkins Oncology Center, Baltimore, MD; David Hockenbery, Fred Hutchinson Cancer Research Center, Seattle, WA; Jonathan Ashwell, Department of Health & Human Services, NIH, Bethesda, MD; Tadatsugu Taniguchi, Inst. for Molecular and Cellular Biol., Osaka University, Osaka, Japan; Douglas R. Green, La Jolla Inst. Allergy and Immol., La Jolla, CA; Steven W. Sherwood, Standford University, Stanford, CA; Peter H. Krammer, Institute for Immunology and Genetics, German Cancer Research Center, Heidelberg, Germany; Alan R. Eastman, Dartmouth-Hitchcock Medical Center, Dartmouth Medical School, Hanover, NH.