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DeVita Dissatisfied With Cooperative Groups On Accrual; NCAB To Hear Wittes' Plan In Feb.

"It may well be that the cooperative group system is broken. We've been working on it for years and it is still not accruing patients [as fast as it should to provide timely answers]," NCI Director Vincent DeVita commented at

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In Brief

Eyre ACS President, Freeman President Elect, Kathleen Horsch Chairman; Davis Heads ASTRO

HARMON EYRE, professor of medicine at the Univ. of Utah College of Medicine, is the new president of the American Cancer Society. He succeeds Virgil Loeb. Eyre is director of cancer education programs at the university and also is director of clinical research for the Utah Regional Cancer Center. **Kathleen Horsch** of Minneapolis was named chairman of the ACS Board of Directors. **Harold Freeman**, director of surgery at Harlem Hospital Center and associate professor of clinical surgery at Columbia Univ., is the new vice president and president elect. . . . **LAWRENCE DAVIS**, chairman of the Dept. of Radiation Oncology at Montefiore Medical Center/Albert Einstein College of Medicine, was elected president of the American Society for Therapeutic Radiology & Oncology at the society's recent annual meeting. Other ASTRO officers are Robert Edland, chairman of the board; James Cox, immediate past chairman; Carl Bogardus, treasurer; Morris Wizenberg, secretary; and Carolyn Freeman, Sarah Donaldson, Frank Wilson, Lester Peters and Robert Greenlaw, members at large. . . . **WINNERS** of the 1987 Albert Lasker Medical Research Awards, presented today (Nov. 20) in New York, are Susumu Tonegawa, Philip Leder and Leroy Hood in basic medical research, and Mogens Schou in clinical medical research. . . . **CANCER RESEARCH** Institute Award for Research in Immunology was presented earlier this month to Rolf Zinkernagel, Univ. of Zurich, and Thierry Boon, director of the Lundwig Institute for Cancer Research in Brussels. Lewis Thomas, president emeritus of Memorial Sloan-Kettering Cancer Center, received the William B. Coley Award. . . . **CLARISSA WITTENBERG** is the new science writer in NCI's Office of Cancer Communications, with the primary assignment of helping Director Vincent DeVita prepare congressional statements, speeches, budget justifications, etc. She replaces Eleanor Nealon, who became chief of OCC's Reports & Inquiries Branch earlier this year.

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Wittes Says Group System Isn't Broken, DeVita Says It May Be

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the National Cancer Advisory Board meeting this week during a presentation on problems in clinical trials and possible ways to overcome them.

Robert Wittes, director of the Cancer Therapy Evaluation Program in the Div. of Cancer Treatment, summarized the months of discussions he and his staff have been having with the clinical cooperative group chairmen and others involved in clinical trials. The primary problem: slow patient accrual.

Wittes described proposals that have evolved on how to address the problems, including more intergroup collaboration and intergroup studies, efforts which have been substantially increased during the past year.

"The group system is not broken," Wittes said. "In fact, some groups are quite good."

DeVita challenged that view with his statement that it "may well be broken," and pointed out that it is costing NCI $55 million a year.

As one example of why he feels the system is not broken, Wittes cited the Cancer & Leukemia Group B adjuvant therapy for breast cancer protocol, started in January, 1985 with a goal of accruing 1,150 patients by October, 1989. Wittes said that it appears now accrual will be completed by December, 1988.

"Why is that a success?" DeVita asked. "With 100,000 patients a year, why shouldn't accrual be completed in one year? I see that as a failure."

Board member Helene Brown suggested that even after completion of accrual, more time is required for followup before results of a study can be determined.

"The fact is, in breast cancer, if we could accrue all the patients needed in one year, within 18 months you would usually know all you would at 10 years," DeVita said. Board member Bernard Fisher, chairman of the National Surgical Adjuvant Breast & Bowel Project, agreed. "That is absolutely correct. In all of our studies, we could see trends in the first year, sometimes in six months."

"If you could answer the questions quickly and then move on, you could build on that information and ask new questions," DeVita said. "We don't need 10 years if patient accrual is faster."

Wittes mentioned a few other successes, when accrual has been on or ahead of schedule. He also referred to an intergroup melanoma study, which was scheduled to accrue 1,400 patients between May, 1983, and December, 1987. The completion date now has been extended to November, 1993, and "that is completely unsuccessful," Wittes said.

"The point Vince made is that even when we are on schedule, that is too slow. We're not beginning to tap the potential," Wittes said.

Wittes described various proposals for improving the system which were discussed last summer with cooperative group chairmen (**The Cancer Letter**, Aug. 21). He will meet again with the chairmen Nov. 30 (NIH Bldg 31, Conference Room 10, 9 a.m., open) to hammer out details for an overall plan to be presented to the DCT Board of Scientific Counselors at its meeting in February.

Basically, the plan would involve "maximizing" the existing network of groups, affiliates, Community Clinical Oncology Program, Cooperative Group Outreach Program, and clinical cancer centers; and expanding the network, primarily to bring in more community participants.

Maximizing effectiveness of the existing network would include steps to simplify protocols, diagnostic tests, data collection, "and make it as easy as possible to participate," Wittes said. Also, the ratio of eligible patients available to participants to those actually entered would be monitored closely, with minimum performance standards established.

Increased funding also would be made available, "although we haven't heard much about that from the group chairmen," Wittes said.

Finally, "technological fixes," such as videotaped informed consent and computerized protocol prompts, would be made available.

"That's optimizing the network, which could increase accrual by a factor of two," Wittes said. That's not enough, and to get any substantial further increase, the network would have to be expanded. That would include efforts to involve more universities, more community hospitals, HMOs and non-HMO for profit providers.

"It's inconceivable that we can have optimal participation without major involvement of communities," Wittes said. Community hospitals and physicians not already participating as formal group affiliates, CCOPs or CGOPs, would be encouraged to affiliate informally. Funds

would be made available to group chairmen to pass on to to participants on a per patient entered basis.

Direct affiliation with CTEP is also a possible way in which the network can be expanded, Wittes said. An example: the network of centers which were quickly lined up and funded to do the interleukin-2/LAK cell trials.

The NCAB meeting was running behind schedule, with little time for discussion by members. Chairman David Korn suggested that "a well structured program" be presented at the February meeting, and the members agreed.

NCAB To Recommend Improvement In Reauthorization, Cancer Funding

The National Cancer Advisory Board will include in its biennial report to the President and Congress its opening shot on renewal of the National Cancer Act.

The Act, with the special authorities that were so important in launching the National Cancer Program in 1971, and without some of those authorities which were chipped away in subsequent renewals, will be included in biomedical research reauthorization legislation due for congressional action next year.

The most critical issue in reauthorization, other than defending the entire Act from attempts to kill it completely, is the matter of "apportionment." In the original Act of 1971, the bill said that appropriations "shall" be made directly to NCI. One of the renewals changed "shall" to "may," and when the Office of Management & Budget decided to take advantage of that miscue, NCI lost much of its flexibility. OMB has decreed that money may not be reprogrammed by an NIH institute unless a corresponding reprogramming is made elsehwere to maintain the original overall level in that program. Exceptions can be made only with approval of the NIH director and OMB.

"That means we have to go across the street everytime we want to do something," DeVita said at a meeting of the Board's Planning & Budget Committee this week. "The Cancer Act was brilliantly conceived. . . If we get all those resources but our hands are tied, we look like damn fools."

Committee members also agreed to include budget problems in the biennial report, including such items as consequences on the Year 2000 goals of not receiving the bypass budget requests; impact of funding cutbacks, such as paying grants at less than 100 percent of recommended budgets; absence of construction funds; insufficient funding for training and intramural programs; and lack of increase in the cancer control budget.

"I would like to make the case that absence of construction funds is worse than problems with the operational budget," Board member John Durant said. "The state of the infrastructure is deteriorating."

THe White House has always opposed construction funding, and Congress in the last few years has not come close to including enough to meet even the minimal needs of cancer research facilities. Both houses this year specifically excluded construction from any funding, pending completion of a survey of national research facilities requirements.

Armand Hammer, chairman of the President's Cancer Panel, did not attend the NCAB meeting this week but sent a statement which was ready by Panel member William Longmire. In it, Hammer suggested that biomedical research reauthorization this time be for a five year period, rather than the customary three.

Board member Enrico Mihich suggested that seven years would be even better. Board member Helene Brown said, "Let's ask for seven and settle for five."

"Like we do on our grants," Mihich said.

NCAB NOTES: This week's meeting was the annual program review, when the update on cancer survival based on the SEER statistics is usually presented. However, DeVita was not happy with the format this time and sent it back for revisions. It will presented at the Board's meeting in February. One improvement it will include: A three-four percent drop in premenopausal breast cancer mortality. . .

The NCAB Public Participation Hearings, held so far in Los Angeles in September and Atlanta earlier this month, have been resounding successes. They were well attended, well received by the public and scientific communities, and had massive media coverage. Helene Brown was in charge of the L.A. meeting, Lois Sullivan in Atlanta. Next two will be in Dallas, with Nancy Brinker in charge, and Philadelphia, headed by John Durant. . .

DeVita, after criticizing the GAO report which was lukewarm on NCI survival figures: "I don't feel restrained on commenting about GAO. Their reports are always negative."

DCBD Board Likes OSP But Rejects RFAs; Two Contract Concepts Okayed

Organ Systems Program working group members, coordinating center Director James Karr and NCI OSP staff members have felt that since reorganization of the program four years ago, their toughest hurdle in clearing concepts they develop through NCI has been the Div. of Cancer Biology & Diagnosis Board of Scientific Counselors.

The DCBD Board has been just as hard on concepts generated by division staff, especially when they involve RFAs, which require allocation of specific dollar amounts and require use of ad hoc reviewers rather than the chartered study sections.

If there is one bastion remaining among NCI advisors of the pure "leave it to the creativity of the scientists and don't try to direct research" school, it is the DCBD Board. The members believe that RO1 and PO1 grants are all that is needed, except for an occasional contract.

When DCBD Director Alan Rabson scheduled three Organ Systems concepts for his Board at its meeting this month, including two RFAs (request for applications), he thought it would be a good idea to give the program's leaders an opportunity to explain their rationale for RFAs and program announcements in cancer biology. Karr came to the meeting, along with Leonard Augenlicht, James Jamieson, Kenneth McCarty, Donald Tindall and Bert Vogelstein.

Jamieson noted that it is difficult to work with solid tumors; that each organ has unique features; and that working groups provide perspective with a multidisciplinary approach. The program attempts to bring new investigators into difficult fields, and it emphasizes the importance of workshops to bring people together and develop collaborations.

Tindall described how the working groups bring diverse groups together, provide model systems, access to tumors and sera, and said the program got him into research that he might never have done otherwise.

When PhDs from molecular genetics get together with clinical investigators, they are encouraged to focus their efforts into clinicall relevant work, Tindall said. Many of the people working with transgenic mice, for instance, are really interested in them to understand gene regulation, but the working group tries to apply them to clinically related model systems.

Board members were impressed with the program's workshops and multidisciplinary approaches, but they questioned the need to develop concepts and their involvement with funding mechanisms. They felt that getting scientistis of different disciplines together would lead to development of research ideas and collaborations to carry them out. The individuals and collaborators could seek their own funding through traditional sources and should not need the more targeted approach through RFAs, they argued.

That philosophy prevailed when the concept proposals were considered. The Board rejected one proposal which would be supported through an RFA, approved another only after it was changed from an RFA to a program announcement (PAs are intended to stimulate RO1 or PO1 applications, which are reviewed by regular study sections), and approved another program announcement without dissension.

The Board also approved recompetition of two resource contracts for the division.

The concept proposals and outcome follow:

Role of circulating biologic markers in detection of subclinical metastasis of breast cancer. Proposed as an RFA, with $4.6 million set aside for total costs to support three to five grants for five years.

Board members were not convinced the study would ever yield the information desired. Stephen Baylin said he felt that it would be very difficult to do and questioned the choices of markers. However, all members agreed the goal was important.

The motion to approve the concept as an RFA was defeated 10-0. When McCarty said he didn't really care whether the concept was approved as an RFA or PA, members voted 7-1 with two abstentions to approve it as a program announcement.

The objectives of this project are to evaluate whether changes in levels of specific, circulating biologic markers can now serve as accurate evidence of subclinical breast cancer metastasis; to determine which individual markers may be most valuable for inclusion in a potential panel of markers useful for detecting such metastasis; and to explore whether changes in levels of such markers soon after primary diagnosis and intervention may also be able to detect residual systemic disease and thus predict probable recurrence. The ultimate goal would be earlier detection, or even prediction, of micrometastasis and recurrence; if that goal can be achieved, better strategies could then be developed for selection of patients most appropriate for adjuvant chemotherapy,

The speed with which breast cancers may metastasize and kill the host varies greatly; some patients may remain cured for many years after initial treatment, while in others the tumor metastasizes and proves fatal within a short period of time. It would be of great value to be able to predict which of these paths a tumor will take, or at least to be able to detect incipient metastasis at very early, subclinical stages.

Several biologic markers in circulating blood have now been reported useful in detecting subclinical metastasis of breast cancer and in monitoring the

course of the disease during treatment. Polyclonal antibodies against breast epithelial cell surface fragments, from human milk fat globule membrane, detect breast epithelial antigens carried in the circulation of breast cancer patients with primary tumors, and in patients in relapse after initial treatment. Early detection of relapse has been obtained with these polyclonal antibodies, sometimes several months before manifestation of any clinical symptoms. Monoclonal antibodies have confirmed that breast cancer cells release into the circulation several of their cell surface components, with molecular weights ranging from 43,000 to more than 400,000 daltons. Assays for these components have been found useful in monitoring the course of breast cancer and the response of the disease to therapy.

Elevation in the circulation of glycosyltransferase enzymes has been reported for various cancers, including primary or metastatic breast cancer, with positive correlations evident between changes in the serum levels of this enzyme and the course of the disease during treatment. Prognostically, decrease of the enzyme levels to normal after surgery was frequently observed in stage 1 breast cancer (83%) but far less frequently in patients with four or more positive nodes (25%); enzyme levels that increased after surgery predicted early pulmonary metastasis in a few patients with no positive nodes.

Oncofetal antigens have also been measured in sera of breast cancer patients. Increased levels of carcinoembryonic antigen (CEA), the one most widely used, have been detected in a certain percentage of breast cancer patients, and have been reported to have short term predictive value. Incomplete synthesis of blood group substances in cancer tissue has resulted in serological alterations that were also able to indicate the presence of malignancy.

The Thomsen-Fredenreich, or T and Tn, antigens represent an early stage in the pathway of symthesis of the NM blood group oligosaccarides, and normal subjects have natural antibodies to these antigens. The blood group substances are not synthesized to completion by carcinomas, and T and Tn antigens have been found to be expressed at higher levels. The circulating, natural antibodies are neutralized by these antigens, and lowered levels of anti-T were also associated with the presence of carcinoma.

There is presently too little information about any of the relevant biologic markers to justify their use in determining breast cancer treatment at any stage. Additional, prospective studies are needed to explore and compare the potential utility of such markers in detection of vary early, subclinical metastasis and/or in prediction of subsequent recurrence by identifying patients likely to be harboring residual disease. The purpose of this research initiative would be to invite investigators with expertise in this area to undertake new cooperations toward such explorations and comparisons. Suggestive and potentially important results have been reported, and it is now timely and appropriate to facilitate methodical testing, either in independent or in collaborative studies, of markers showing promise for detecting early, subclinical metastasis.

The intent of the concept is to emphasize markers that would not need to be breast cancer specific, but that would closely reflect the tumor burden of the host, i.e., assays highly sensitive for detecting early subclinical metastasis and for correlating changes in the levels with changes in the clinical course of the disease. For this reason, the study would preferably cover a broad spectrum of disease stages and tumor burdens rather than just one particular stage of breast cancer, and could include comparisons with marker levels in normal women with benign breast diseases. Markers to be considered could include breast epithelial antigens, glycosyltransferases and related enzymes, oncofetal antigens, transplantation antigens, or blood group antigens. Correlations between different antigens and their comparison in diagnostic and prognostic studies would be appropriate and potentially important. Validation might well aim to include patients exhibiting a broad range both of baseline levels and of nature and extent of fluctuations.

The intent is to encourage collaborative studies or a possible network of laboratories with define expertise in biologic marker assays, to facilitate comparisons of results with different markers and perhaps eventual selection of a panel of the most predictive markers. Investigators might wish to plan for validation of their observations by others, and a system for this could be proposed. Such collaborations could develop uniform procedures for collection, storage, etc., and might also be of value in helping to accrue into the study sufficient numbers of patients. It is anticipated that awards would be made for five years and that patient accrual could continue through at least three of these five years, which would provide for two to four years of followup. Recurrence would be the principal and focused endpoint for comparing the prognostic value of the markers; the collaborative studies could choose a uniform definition of recurrence and appropriate procedures for its confirmation. Comparison with other prognostic variables, such as estrogen and progesterone receptors, might help to determine whether the markers under study are able to contribute independent prognostic information beyond what is already being collected and applied in clinical practice.

Regulation of prostatic involution as related to prostatic cancer. Presented as a program announcement and approved by a 10-0 vote after Tindall, who made the presentation, explained the rationale for using a rat model.

The four major objectives are to understand the nature of both the morphological and functional heterogeneity of the ductal-acinar network in the intact prostate; to study the regulation of gene expression during prostatic involution, including hormonal regulation of stimulated and repressed genes, nonhormonal factors such as extra cellular matrix and growth factors, and cell-cell interactions which may affect specific genes; to study the activities and functions of specific gene products in the prostate following androgen deprivation and the nature of their substrates; and to study the biochemical properties and genetic regulation of cells in the prostate following involution.

Standard treatments to block cell proliferation in fast growth tumors cannot be utilized effectively in prostate cancer because of the slow growth of these tumors. In addition, prostate cancer is usually androgen sensitive but not androgen dependent, rendering treatment by androgen blockade only partially effective. Because of these limitations a new approach must be explored in order to understand how one might enhance cell death in these tumors. The focus of this concept, therefore, is to address the biological mechanism of of prostate involution. Since it is becoming increasingly obvious that treatment of prostatic carcinoma by androgen blockade, whether by castration, or by more elaborate therapeutic combinations, is only partially successful at best, the elucidation of the detailed mechanism of prostatic involution should herald a second generation of therapies designed to enhance the rate and degree of involution and to ensure the continued suppression of growth. The prostate is particularly suited for this kind of research, since many elements of the process have been described in some detail already. A concerted effort

to unravel the remaining links in the processes which control involution in the prostate may lead to new ways of treating prostate cancer.

The following examples of possible approaches are not all inclusive. It is not implied that any single applicant should pursue all or any of these examples. Other novel approaches with appropriate rationales are encouraged:

Nature of morphological and functional heterogeneity with prostatic ductal networks in normal and regressing prostate.

The cellular and functional heterogeneity of the prostate has recently emerged as a major feature of the gland. The pathology of the prostate gland may be a reflection of the underlying heterogeneity of the normal prostate. While the importance of heterogeneity is now accepted, a more detailed description is now required which incorporates a broad array of perspectives. At the morphological level the histological and cytological differences of epithelial and stromal cells must be defined at both the light and electron microscope levels. To explore regional heterogeneity within the prostate, microdissection procedures must be used to display the gland as a two dimensional array of ducts. In preparations of this kind differences in anatomy, function, growth and involution can be detected among prostatic lobes, and ducts in a single lobe, as well as between the proximal and distal portions of a single duct. This technique will reveal the true architectural complexity of the prostate, its pattern of ductal branching and the changes that it undergoes in response to androgen deprivation. Using a battery of immunologic probes, it will be important to define those regional areas where specific activities are expressed. Biochemical methods used to study prostatic growth, hormone sensitivity, and function usually require tissue homogenization, and histological analyses have routinely been done on random sections. Neither approach can adequately address the question of regional heterogeneity within the prostate, especially as it relates to involutional changes due to androgen deprivation. Advances in understanding of castration induced prostatic involution will have to take into account the fact that different zones of the prostate behave in radically different ways to androgen deprivation.

Examination of prostatic involution should utilize tumor models and also the normal prostate in situ. Clearly, in both cases, the models will have to account for mixed cell populations (androgen dependent and androgen independent) and, in the case of the prostate in situ, will have to address distala vs. proximal ductal architecture, which responds differently to androgen deprivation. It will be important to determine why distal ducts involute completely in response to castration while proximal ducts merely atrophy but are maintained. To answer this important question each of these areas will have to be studied separately, making analyses of total prostatic homogenates an entirely inappropriate model.

Taking into account the reginal heterogeneity within the prostate, new immunological, cDNA probes and protein markers indicative of epithelial regression could be developed and added to morphological markers of regressive change. Regression could be defined in metabolic, biochemical and molecular terms, and the possible role of cell-cell interactions in this process will need to be determined.

Some questions that might be answered regarding the morphology and function of the normal prostate are:

1. Where within the ductal networks are specific secretory products expressed?

2. To what extent do urothelial cells from the urethra extend distally into the prostatic ducts?

3. What is the distribution of basal cells along the proximo-distal extent of prostatic ducts, and does it correlate to regional differences in biological activities such as DNA replication or secretion?

4. How does the stromal/smooth muscle organization vary proximo-distally?

5. Does basement membrane turnover vary, along prostatic ducts?

Once the morphological variation within the prostatic ducts has been established, it will be important to assess the changes in the functional and morphological heterogeneity within the prostate during the course of involution. Some questions that could be addressed are:

1. Where along the involuting prostatic ducts are the androgen repressed genes expressed?

2. Where are the proteolytic enzymes expressed?

3. Does the expression of degradative enzymes, such as cathepsin, coincide with regions where complete involution occurs, i.e., the ductal tips?

4. What is the time course and nature of changes in the basement membrane during prostatic regression?

All of the above can be accomplished through immunocytochemical, autoradiographic, morphological, and morphometric studies, using microscale biochemical techniques, which permit direct analysis of biochemical activieis (such as enzymatic activities, androgen, estrogen and prolactin receptor levels, DNA replication and gene transcription) in different regions of the prostatic ducts.

Gene expression during prostate involution

It is now well established there there are a number of genes that are induced in the prostate after castration. There are also major changes in the cytoskeleton and nuclear matrix components of the epithelial (and possibly stromal) cells. In order to fully understand the process of involution, it would be important to identify and characterize these induced genes and to characterize the nature of the gene products. Since changes in the extracellular matrix (ECM) may cause both changes in the cytoskeleton/nuclear matrix and changes in gene expression, it will be important to determine which genes are induced by changes in the ECM, and which genes are repressed by androgens. In order to accomplish this, it will be necessary to characterize the alterations in the basement membrane, cytoskeleton and nuclear matrix, which occur during the early stages of prostate involution. In this way, through a detailed analysis of the temporal changes in ECM structure, cytoskeleton and nuclear matrix associations and changes in gene expression that occur during involution, it will be possible to identify components which initiate the process and those which are altered as a consequence of the process. There are several additional areas in which research is needed to elucidate the mechanisms of prostate involution. These include:

1. Identification and characterization of androgen repressed genes in the prostate. The characterization should include the kinetics of the induction process and the relationship of the induction to androgen receptor levels. The nature and activity of products of these genes also needs to be investigated. This can be achieved by biochemical techniques including two dimensional gel electropheresis to identify all proteins which are uniquely produced by the prostate during involution. Purification of the proteins of interest and the preparation of appropriate antibodies will provide the necessary tools for immunocytochemical studies designed to localize the site of production of these proteins within the ductal architecture of the prostate cland as well as to determine whether the expression of these genes is restricted to one cell type.

2. Characterization of the alterations in the basement membrane after castration. These investigations should also elucidate the regional differences

in the basement membrane in the ductal acinar networks of the prostate using the microdissection techniques with recombinant methods (such as in situ hybridization) and morphometric methods.

3. Characterization of the changes in the cytoskeleton which occur after castration. These studies could focus not only on the changes in composition of the cytoskeleton but also on the anchorage points of the cytoskeleton to the basement membrane, since at least one integral membrane protein (integrin) has been proposed as a substrate for one of the proto-oncogenes (c-scrc).

4. Characterization of the changes in the components of the nuclear matrix and nuclear envelope. These studies could also be designed to elucidate the nature of the interactions between the androgen and estrogen receptors and the chromatin associated with the nuclear matrix.

5. Characterization of the effects of changes in the composition of the basement membrane or cytoskeleton on gene expression. The major obstacle to this research lies in the need for a good cell culture system. The effect of the components of the extracellular matrix on gene expression must be dissected in culture. While it is clear that the ECM can be viewed as being permissive to androgen regulation, there is as yet no optimized cell culture system for prostatic epithelial and/or stromal cells. Since the analysis of the events controlled by ECM requires such a system, there is a need for a well characterized cell culture system for studying the control of gene expression in vitro.

6. Identification of putative regulatory proteins which are synthesized early in the process of involution and which induce subsequent events in the process. If such regulatory genes are identified, it will be important to study the mechanism of induction of these genes to determine whether it is possible to specifically enhance the rate of cell death by manipulating the expression of these genes.

Characterization of the fully regressed prostate

In view of the poor overall success of antiandrogen therapy, it is becoming increasingly important to analyze the fully regressed prostate to determine what unique features of the gland are responsible for its refractoriness to antiandrogens. These questions are important from the standpoint of prostate cancer because of the subsequent androgen independent growth of many prostate tumors following remission with hormonal therapy. There are a number of important questions which arise, including:

1. Which cells remain impervious to the absence of androgens? What is the biochemical makeup of these cells? To what extent are they regulated by hormonal and nonhormonal factors including extracellular matrix and cell-cell interactions? The fully regressed state should be examined in detail to assess the stem cell capability of the regressed ductal tips vs. more proximal ductal regions. Moreover, the biomorphometric approaches of deKlerk and Coffey, which in random tissue selections show a 10 fold change in epithelial stromal ratio (5:1 to .5:-1), must be reexamined using sampling techniques that allow assessment of the changes in epithelial stromal ratios in proximal vs. distal regions. It will be important to define the cells involved from a morphological approach. A stereological analysis and immunocytochemical localization studies of cell specific markers should identify the cell types remaining following involution as well as their localization within the framework of the ductal networks.

2. What genes are expressed in the fully regressed prostate gland? Are the genes that are expressed regulated by hormones other than androgens (for example estrogens or prolactin)? A biochemical analysis of the intracellular and secretory products of these cells will lay the groundwork for defining their maintenance and regulation. Characterization of the fully regressed tissue should include a full analysis of the proteins and mRNAs present in the tissue. This would include cDNA cloning and the analysis of the steady state mRNA levels of specific sequences. A cDNA library may shed light on which genes are important for maintaining cell function even in the absence of androgens and would also provide an analytical tool with which to assess the effects of other therapeutic modalities.

3. What is the nature of the extracellular matrix in the fully regressed tissue? Both extracellular matrix and cell-cell interactions should be examined in order to define the total environment of these cells and their method of survival in the absence of androgens. Using both biochemical and gene expression as endpoint markers, the influence of nonandrogenic steroids such as estrogens, prolactin, thyroxine and alpha adrenergic agonists should be examined for their effects on these cells.

The problems outlined above require a wide array of techniques and specialties. Researchers using a number of techniques from a variety of disciplines can contribute to these studies. The disciplines will include morphologists, cell biologists, endocrinologists, protein chemists and molecular biologists. Since it is unlikely that any one laboratory has all the requisite skills, multi-institutional and interdisciplinary collaborations would greatly facilitate the conduct of these projects.

Facility for preparing and housing virus infected intact and chimeric mice. This is recompetition of a contract now held by BioQual Inc. A five year award is anticipated at a total annual cost, including direct and indirect costs, of an estimated $410,000 a year.

This contract provides support for in vivo murine immunological research for investigators of the Immunology Branch in the areas of preparing and housing virally infected mice, and the radiation chimeras, as well as gene transfection and embryo transfers in mice. Due to restrictions on the use of infectious viruses and on receiving and housing mice from inapproved sources on the NIH campus, the Immunology Branch has been limited in the types of experiments that can be performed involving infectious agents and the preparation of radiation chimeras using certain recombinant and mutant mouse strains.

This contract currently supports research studies of the project officer, Gene Shearer, and five other senior investigators in the branch--Richard Hodes, Ronald Gress, David Sachs, Alfred Singer and Dinah Singer. To supplement the ongoing research in mechanistic and genetic aspects of immune regulation in mice, experimental protocols of four broadly different categories are being performed by the contract.

First, the role of infectious virus (in particular murine cytomegalovirus (MCMV), is being investigated in different inbred strains of mice. The objective of these studies is to investigate the immunosuppressive and immunopotentiating aspects of MCMV in different mouse strains alone, and when given in conjunction with or in series with other potential immunological insults and stimuli, e.g., allogeneic leukocytes, soluble antigens, or other viruses. Specifically, these MCMV studies are being performed to investigate (a) the syngergistic aspects of graft vs. host reactions and CMV in transplantation; (b) the helper (immunopotentiating) effects of MCMV on cellular and humoral responses to conventional antigens (e.g., TNP ovalbumin, TNP self, and alloantigens) and autoantigens (e.g., DNA, and acetylcholine receptor), as well as to alogeneic tissue grafts; (c) synergistic effects with murine retroviruses; and (d) the susceptibility of mice depleted in vivo of different T

cell subsets to virus infection, and the mechanisms responsible for immune protection against viral infections and pathogenesis.

The second category of research related services performed by the contract is to prepare bone marrow radiation chimeras using donar and/or recipient mice that (due to their source and health status, e.g., infection with Sendai or hepatitis virus) cannot be received and housed in the B2B animal facility. These include the use of donor cell andor irradiated recipients from unapproved animal sources (i.e., sources that have viruses or parasites in their mouse colonies), as well as mice bred in DCBD's own satellite facilities and from the contract of David Sachs.

The third category of research related services provided by the contract is to perform murine embryonic transfers into pseudopregnant female mice.

The fourth category of research related services of this contract is the construction of experimental mice by introduction of foreign genes and DNA into the murine germ line, either by direct microinjection or by the use of retrovirus vectors.

The contractor will be expected to perform the following research support services as directed by the project officer:

Up to 2,500 tail bleedings of mice per year; up to 2,500 intraperitoneal injections of mice per year; up to 2,500 ;intravenous injections of mice per year; up to 1,500 subcutaneous injections of mice per year; embryo transfers in up to 500 pseudopregnant mice per year; up to 500 palpations of mice per year, for detecting tumors; up to 500 subcutaneous thymus grafts in mice per year; whole body irradiation of up to 2,500 mice per year; preparation of up to 2,000 radiation chimera mice per year; preparation of up to 200 ml of murine CMV virus stocks per year; up to 25 plaque assays for MCMV per year; grow and harvest influenza virus from up to 100 chicken eggs per year; up to 25 hemagglutinin assays for influenza virus in egg allontoic fluid per year; sterile preparation of up to 6,000 lympoid cell suspensions per year and deliver to NIH campus; up to 50 tissue cultures of murine lymphocytes for generation of cytotoxic T lymphocytes and assay of these cultures; ELISAs of up to 7,000 serum samples per year for anti-CMV antibodies; manipulationss that include introduction of foreign genetic material, by cell fusions, viral infection, microinjection, etc. in up to 200 embryos per week; preparation of up to 50 vasectomized male mice per year for mating with females for preparation of pseudopregnant mice; provide hormonal treatment of up to 500 female mice per year for preparation of pseudopregnant mice; and mating of up to 500 pseudopregnant mice per year with vasectomized males.

Feral mouse breeding colony. Recompetition of a contract now held by Hazleton Laboratories. The new contract will be awarded for three years, at an estimated total cost of $135,000 per year.

This colony is a major resource for the Oncogenetics Section of the Laboratory of Tumor Immunology & Biology and plays an integral part in the study of the role of mouse mammary tumor virus (MMTV) in the etiology of murine mammary gland neoplasia. This contract also provides mice and tissue samples upon request to other investigators at NIH and other research facilities around the world.

Research on the role of MMTV in the etiology of murine mammary gland neoplasia has been restricted to two or three laboratory strains of inbred mice. In earlier work, this effort was expanded to feral breeding populations derived from different geographical locations. The feral mouse colony has been a major resource to determine whether there are other int loci which contribute either to the development of preneoplastic lesions or mammary tumors. The current contract has focused on three pedigreed outbred colonies of feral mice which have unique characteristics pertinent to this question--Czech II mice, which lack MMTV proviral sequences in their germline; MCPT mice, which are infertile with inbred mouse strains and which express a MMTV related virus in lactating mammary glands and mammary tumors; and MS mice, which are partially fertile with inbred mice. MS females are also infected with an MMTV related virus and develop pregnancy dependent tumors which subsequently progress to pregnancy independent tumors.

A second related focus of current and future efforts is to determine whether the frequency with which individual int loci are activated in mammary tumors is a function of the host genetic background, the strain of virus or a combination of both. To undertake this program, a MMTV free subline of Czech II mice has been developed.

A third aspect of the program which is dependent on the mouse colony is a long term project to develop an in vivo system to directly analyze the biological effects of recombinant DBA vectors containing individual activated into loci as well as combinations of activated into loci or other proto-oncogenes. This program is necessary because of the absence of an adequate tissue culture system and the lack of information on the developmental pathway of subpopulations of cells in the mammary gland. The Czech II mammary gland has recently been reconstituted by introducing enzymatically derived single cell suspensions of mammary tissue back into female mice lacking the mammary gland fat pad. It is proposed to use electroporation to introduce recombinant DNA vectors containing activated into loci and proto-oncogenes to determine their effect on the induction of preneoplastic lesions and mammary tumors. The laboratory in other studies has successfully used electroporation to introduce recombinant DNA into a variety of tissue culture cell lines. A similar strategy will be used to determine the biological activity of these recombinant DNA constructs on hyperplastic outgrowth lines which have been developed in the virus positive Czech II line.

The contractor will provide proper facilities and technical support for the maintenance and breeding of the indicated species of mice, total 1,000. This will include technical help experienced in the handling and husbandry of feral mice, breeding of feral mice and knowledge of requirements for maintenance of outbred colonies; milking mice, observation of mice for early tumor development; surgery and dissection, injections and preparation of tissue for histology.

Markers of colonic stem cells and differentiation was the title of the RFA concept rejected by the Board by a vote of 7-0, with three abstentions. Board members agreed there is a need to develop better ways to culture colonic epithelium and to define culture conditions, but they questioned whether this RFA was the appropriate way to do it. Augenlicht, who made the presentation, said the working group felt that earmarking specific funds would be necessary to get the scientific community to respond.

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