THE CANCER LETTER
RESEARCH EDUCATION CONTROL

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Vol. 1 No. 44

Oct. 31, 1975

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Subscription $100 per year

ENVIRONMENTAL CARCINOGENESIS SEEN AS NEXT BIG GROWTH FIELD; NCAB HEARS PROBLEMS, PRIORITIES

James Peters, director of NCI's Div. of Cancer Cause & Prevention, led off a presentation to the National Cancer Advisory Board on environmental carcinogenesis by making the statement, "Probably no more than 3-4% of all cancers are determined by intrinsic host factors. Therefore to say that most cancers have environmental origin is obvious, if not redundant."

Most NCI executives agree, although perhaps not to that extent, and

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In Brief

COOPERATIVE GROUP INVESTIGATORS TREAT 10 FOR EVERY PATIENT IN PROTOCOLS – HOLLAND

COOPERATIVE GROUPS had 15,000 cancer patients enrolled in their various protocols in 1974. James Holland, chairman of Acute Leukemia Cooperative Group B, believes that investigators in the cooperative groups treated or influenced the treatment of 10 additional patients for every one they treated in group programs . . . . **NCI'S ADVISORY** groups are surprisingly unique among HEW's array of non-government advisors, according to Benno Schmidt. As a member of the Biomedical Research Panel which is taking a hard look at all federally-supported health research efforts, Schmidt was "shocked to learn that we're not typical. Advisory groups in the cancer program have few if any vacancies, appointments are promptly made with no politicization, from a hard-working talent pool. The Board (National Cancer Advisory Board), advisory committees and subcommittees really work. We've found that ADAMHA (Alcohol, Drug Abuse & Mental Health Administration) and others have serious problems in getting and keeping people on their boards". . . . **HOUSE PASSED** the bill extending the National Research Service Awards program for two more years, 375-5—not only a veto-proof margin but so overwhelming that the President probably will not bother to veto it. It authorizes $175 million for the current fiscal year, $200 million for fiscal 1977. The appropriation for 1975 was $175 million, of which NCI received $22.2 million. If the figure in the House bill stands, that means NCI would receive the same amount this fiscal year as last, even if the full authorized amount is appropriated, which isn't likely. NCI had hoped to get at least $25 million this year. Only minor changes were made in the Act, those relating to the pay back requirements. Previous language required recipients to serve specified amounts of time following completion of their training in "health research or teaching." The new language adds, "or any combination thereof which is in accordance with usual patterns of academic employment." Recipients electing to repay in cash rather than service now will be charged interest at rates which reflect prevailing consumer interest rates. The Senate has not yet acted on this bill.

OVER-RIDING NEED: NEW TEST SYSTEM QUICKER, CHEAPER THAN ANIMAL STUDIES

(Continued from page 1)

they are backed by a growing number of scientists around the country. That's why health lobbyists and Congress are becoming more insistent in demanding that the cancer program escalate support of environmental studies. "If more than 90% of all cancers have environmental causes, why is only 10% (or 1%, or whatever, depending on who is talking) of NCI's budget going to environmental carcinogenesis?" they ask.

Director Frank Rauscher usually responds by saying that NCI's money goes where the research opportunities are, that the leads and opportunities in environmental carcinogenesis do not yet exist to support major increases there. But he acknowledges that the situation is changing.

The day-long presentation to the NCAB described some of the problems and the opportunities that do exist, and left little doubt that research into environmental carcinogenesis will be the cancer program's next big growth area.

Peters listed as "some of the major problems we face:"

1. Bioassay systems are difficult, expensive and time consuming. "We must develop more rapid, less costly and more dependable test systems."

2. It is nearly impossible with current knowledge to extrapolate animal data to man. "Therefore, we must look to epidemiological evaluation for better guidance."

3. Using animal data and current methods, "we are unable to quantify those responses in man, and we cannot identify threshold levels, if they exist. We need more work in this area before we can hope to identify safe levels, if indeed we will ever be able to do so."

4. Problems in risk assessment. "Are there high or low risk groups? Can we identify them?"

5. NCI's newly emerging responsibilities to the public, the economy, the legislative body, and other government agencies, specificially the regulatory agencies. "The implications of the toxic substances act must be faced head on. We must become involved in the risk/benefit assessment of our findings.

6. "We have a specific need for increased epidemiological activities to evaluate the impact of the preventive actions we recommend and implement."

Peters said his staff, working with NCAB's Subcommittee on Environmental Carcinogenesis, is "formulating a series of action recommendations" which will be presented at a future Board meeting.

Peters suggested that the emphasis will be on environmental factors which affect human food and water supplies because that is where the predominant exposure of man to potential carcinogens exists. "The guts, the lung, and the skin are the major portals through which external molecules exchange with the internal milieu," Peters said. "The GI tract largely exceeds in surface the other exchanging systems. This difference is true also if we consider the low molecular density of matter impinging on the lung and the skin, and the high density of that impinging on the GI tract."

Considering these factors, "then the relative positions of gut, lung and skin (in exposure) are approximately one million to one thousand to one," Peters said. He told the Board to keep those figures in mind "because they will help you understand the epidemiological and experimental priorities."

Peters noted that viral oncology offers two complementary theories, both of environmental significance.

"Resident cellular oncogenes may be the ultimate targets on which environmental insults act to trigger the transformations necessary to initiate cancers. Other viruses, to be considered true environmental agents, may have an intrinsic and infectious capacity to trigger transformation. The Papova viruses are capable of doing so in animals, although their hazard as human cancer causes has not been yet demonstrated," Peters said.

David Clayson, Eppley Institute, discussed bioassay, the mode of action of carcinogens and anticarcinogenic approaches.

"From what we know today, it is dangerous to assume that a chemical which induces cancer in animals will not do so in man, unless there is convincing evidence to the contrary," Clayson said.

"The design of a bioassay involves a series of compromises. . . Essentially, most carcinogens are relatively unreactive chemicals which require activation by the metabolizing enzymes of the liver or other tissues to highly reactive entities which then interact with certain chemical groupings in the tissues, to give rise to latent tumor or transformed cells. . . Some carcinogens are sufficiently reactive not to need metabolic activation, for example biological alkylating agents such as the nitrogen mustards. A few, such as asbestos, may not necessarily need to interact in the same manner as the more conventional carcinogens.

"Test species should be able to activate the potential carcinogen in as similar a manner to man as possible," Clayson continued. "This is difficult to ensure," due to differences between species in such factors as immunological competence, ability of a species to repair its own genetic material through the various DNA repair mechanisms, lifespan, and many others.

Those difficulties "are completely overshadowed" by the costs of bioassays in forcing scientific compromises, Clayson said. The fact that there are now 24,000 to 30,000 different substances in the technological environment, and 500 to 700 new ones are introduced each year, makes it necessary to devise more economical testing forms. The simplest bioassay costs $100,000, he pointed out.

"For this sum, one gets an indication whether the substance induces tumors in experimental animals and therefore potentially is capable of so doing in man. It is probably adequate for the purpose and to date, seems capable of demonstrating most substances which are known to induce cancer in man. The possibility that it will miss weak carcinogens, which will subsequently be demonstrated in man, cannot be excluded."

But further compromises exist, Clayson suggested. Oral administration of the test compound is preferred, although this may not be the common route of exposure for man. Animals show different sensitivities to certain carcinogens at different stages of life, he pointed out, but NCI guidelines ignore that. FDA seems to be moving in favor of two-generation tests in which one generation is treated from conception to death. "It is too early to predict whether the two types of test will give different results at a qualitative level. It does seem possible, however, that this difference in protocol will ultimately lead to duplication in testing, a tragic waste of much-needed resources.

"There is still a great need for further research into testing methods. I do not believe we have any reason to be satisfied with present testing protocols. We do not know how to choose the most suitable model for metabolic activation, let alone for the other biological variables. Even more important, we are only just developing reasoned methods for the selection of chemicals for test.

"We shun the question of which substances should be tested by NCI and which should be tested by those who make and use them and, hopefully, expect to profit by them," Clayson said.

Clayson commented that cocarcinogenesis is the situation in which a noncarcinogenic substance enhances the efficacy of a carcinogen, and that anticarcinogenesis is the reverse. "It is important to note that removal of a cocarcinogen may be as effective as, and a lot more practical, than trying to eradicate the last traces of a widespread environmental carcinogen or the use of an anticarcinogen." Anticarcinogenesis is still in its infancy, Clayson agreed, although "its potential is considerable." Examples include enzyme inducers, which are among the best-studied anticarcinogens, and vitamin-enzyme co-factors, antioxidants, and retinoids.

James Miller, McArdle Laboratory, discussed the metabolic activation of chemical carcinogens and how this relates to mutagenicity. Testing chemicals for their mutagenic effect is much faster and less costly than current in vivo methods, and could possibly be the quick and inexpensive method so badly needed for carcinogenic screening.

"Studies in the past decade by which chemical carcinogens produce cancer have demonstrated that the great majority of the known chemical carcinogens are not carcinogenic as such but are really precarcinogens," Miller said. "These precarcinogens must be converted metabolically in the body to reactive ultimate carcinogens, sometimes with the formation of intermediate proximate carcinogens. Inactivation occurs at every level and the balance between the activation and inactivation pathways is an important factor in the rate of tumor formation. . . .

"It's at the level of the ultimate carcinogenic forms that common properties of chemical carcinogens make themselves evident," Miller continued. "Despite the variety of structure of chemical carcinogens, their reactive and carcinogenic ultimate forms are all electrophiles and as such possess carbon and sometimes nitrogen atoms that are electrophilic or electron-deficient. The significance of this is that these electron-deficient species seek out electron-rich or nucleophilic nitrogen, sulfur, oxygen, and sometimes carbon atoms in cellular components and combine with them non-enzymatically to form carcinogen residues covalently attached to informational macromolecules such as nucleic acids and proteins in the cell. . . .

"It appears reasonable that some of these bound forms initiate the multistep process of carcinogenesis and lead eventually to tumor formation," Miller said.

"A number of mechanisms can be envisaged—for example, genetic ones in which mutations or heritable changes are caused by the carcinogen bound to the DNA and epigenetic mechanisms in which carcinogen bound to certain proteins might cause an abnormal expression of information in the DNA—and either of these general mechanisms might operate through the expression of carcinogenic viral information already in the DNA. . . .

"This is their mutagenic activity since wherever it has been possible to bring ultimate carcinogenic forms into contact with DNA in appropriate test organisms—usually bacteria and other microorganisms—mutagenesis can be observed, that is, the occurrence of heritable changes in the properties of the organisms," Miller said.

Miller concluded that "under the right metabolic conditions chemical carcinogens are mutagens. Thus the mutagenicity of the active forms of chemical carcinogens provides the basis for the use of mutagenicity tests combined with metabolic activation for the detection of potential carcinogens in our environment."

Arthur McGee, Stanford Research Institute, reviewed the five-year-old NCI-supported effort in which SRI developed a method for ranking untested chemicals for accession to carcinogenicity bioassay and subsequently acquiring and analyzing information on chemicals.

SRI's work supports NCI's Chemical Selection Working Group and selection committee; aids the Cancer Control Program by identifying known carcinogens and assists in the production of monographs that will recommend control and prevention programs; assists the International Agency for Research

on Cancer in the selection of chemicals for study.

SRI has developed a set of criteria for chemical selection. The most important, McGee said, is the production and exposure of humans to chemicals. Of 2 million known chemical substances, only about 30,000 are produced in commercially significant quantities.

Other criteria:

Present amount of human exposure; projected future amount or increased use of the chemical; determination if the chemical is declining in use or if there is an available substitute; evidence of carcinogenicity from previous tests; suspicion of carcinogenicity because of a chemical's structural relationship to known carcinogens; physical-chemical properties which could give an alert to suspected carcinogenicity; mutagenicity properties; suspected interactive effects such as promotional, cocarcinogenicity, etc.; existence of families or groups of chemicals with high human exposure but little or no carcinogenicity testing; existence of epidemiological clues that associate exposure with high cancer incidence rates; degree of regulation presently imposed upon a chemical; and the ability to take corrective actions if the substance is demonstrated to exert carcinogenic effects.

"The present regulatory status must be checked," McGee said. "A chemical presently under close control for reasons other than carcinogenicity should not require testing for carcinogenicity. Similarly, if control action is infeasible, the decision to test the chemical should be questioned."

So far, SRI has investigated these exposure modes—air pollutants, OTC drugs, water pollutants, cosmetics, intentional food additives, soaps and detergents, pesticide residues in food, trade sales paints, and prescription drugs. SRI has identified 3,200 chemicals found in 900 product types, representing 18,000 chemical-product combinations.

Three new exposure categories are under development—agricultural chemicals, adhesives and sealants, and occupational exposure.

CONFEREES GIVE NCI $743.5 MILLION PLUS TRAINING FUNDS FOR 1976

House and Senate conferees working on the HEW appropriations bill for the 1976 fiscal year have settled on $743.5 million for NCI, not including from $22-25 million that will be appropriated later for training programs.

None of the figures in the bill have been announced publicly, since the conferees are still debating the controversial antibusing amendments. But ***The Cancer Letter*** has learned that conferees decided to give NCI 40% of the extra amount voted by the Senate over the House figure.

The House had voted $703.5 million for NCI, the Senate $803.5 million, less training funds in both cases.

With the training money, NCI's total would be about $775 million, $5 million less than NCI staff had anticipated in drawing up 1977 budget requirements (***The Cancer Letter***, June 27). The estimate then was that with $780 million, NCI could fund 56% of approved renewal grants and 48% of approved new grants.

ABSTRACTS OF PAPERS FROM COPENHAGEN SYMPOSIUM ON RESEARCH ON LEUKEMIA

The VIIth International Symposium on Comparative Research on Leukemia and Related Diseases was held Oct. 13-17 in Copenhagen. Abstracts of certain papers presented at the symposium appeared last week in ***The Cancer Letter****; others follow below. The International Assn. for Comparative Research on Leukemia and Related Diseases will publish the complete papers by mid-1976. Copies may be obtained by writing to David Yohn, IACRLRD Secretary General, Ohio State Univ., 1580 Cannon Dr. Suite 357, Columbus, Ohio 43210.*

Interaction Between Chemical Carcinogens, Oncogenic Viruses, And 17-β-Estradiol — ***James Blakeslee, David Yohn, George Milo, & Ronald Hart, Ohio State Univ.***

Individuals with Xeroderma pigmentosum (XP), a rare inheritable disease, exhibit a higher frequency of malignancy than normal individuals when exposed to sunlight. Fibroblast cells from these individuals are deficient in repair of UV-induced DNA damage. Cells from such individuals, however, are not more susceptible to SV40 transformation, than are cells from normal individuals. However, the frequency of SV40 transformation of normal human cells can be enhanced by pretreating with a chemical alkylating agent such as N-Methyl-N'-Nitro-Nitrosoguanidine.

A tumor promoter, phorbol myristate acetate ester, has been reported to inhibit excision DNA repair and enhance semi-conservative DNA synthesis. Our studies indicate that 17-β-Estradiol has similar biologic activities depending upon concentration. Accordingly we have investigated the synergistic interactions of 17-β-Estrodiol, and various chemical carcinogens that produce selected forms of DNA damage on transformation of human cells by Feline Sarcoma Virus (FSV) and by SV40. These studies revealed that:

1. Chemical carcinogens producing genetic damage of the x-ray type, e.g. MNNG, preferentially enhanced viral transformation when applied prior to infection.

2. Carcinogens producing damage repaired by both x-ray and UV-type repair e.g. Benzo (*a*) Pyrene, enhanced transformation when applied before or after infection.

3. Concentrations of 17-β-Estradiol, which stimulated semi-conservative DNA synthesis and inhibited unscheduled DNA synthesis, enhanced transformation independent of chemical carcinogens.

4. Similar concentrations of 17-β-Estradiol acted synergistically with DNA damaging chemical carcinogens to produce the greatest enhancement of virus transformation.

The results indicate that 17-β-Estradiol can function as a promoter of virus transformation of human cells at concentrations which inhibit excision repair and stimulate semi-conservative DNA synthesis, but not at those concentrations which inhibit semi-conservative synthesis.

A Murine Virus-Induced Leukemia Involving Two Subpopulations Of Lymphocytes – ***Peter Dawson, Steven Dresler & A. Howard Fieldsteel, Univ. of Oregon***

In contrast to human lymphocytic leukemias, the majority of which involve B cells, most murine lymphatic leukemias are thymus dependent and composed of T cells. However, the lymphatic leukemia virus (LLV) obtained from Friend virus produces disease in both intact and thymectomized mice. We report the morphology and cell markers of such leukemias.

BALB/c mice were thymectomized at 3 to 5 days of age. One group received 0.1 ml. ip of rabbit anti-mouse thymocyte serum (ATS) on days 8 and 10 and was inoculated with LLV on day 9. A second group was inoculated with virus on day 60 and given o.2 ml. ip of ATS on days 58, 60, 62 and 64. Thymic and spleen cells from leukemic animals were tested for the presence of C3H θ antigen and immunoglobulins at the cell membrane using an indirect immunofluorescent method.

In 12 of 14 intact and sham thymectomized mice, the thymus was involved microscopically and the leukemic cells infiltrated the thymus dependent areas of the lymphoid tissues. In 8 animals, the leukemic cells had demonstrable θ antigen and immunoglobulins. All 14 leukemias were considered to be primarily T cell.

In contrast, the leukemic cells of 12 virus inoculated, thymectomized, ATS-treated mice showed a virtual absence of both θ antigen and immunoglobulin at the cell membrane. Morphologically, the leukemic cells had a different distribution involving primarily the splenic red pulp and sparing the periarteriolar sheath. The superficial lymph nodes were uninvolved and showed paracortical depletion. These data suggest that the leukemias were not of T cell origin.

Cell-free extracts of individual spleens from three thymectomized leukemic animals were inoculated into intact newborn mice. Extracts from two of the spleens produced typical T cell leukemias. All the leukemic involvement of the thymus was total in 5, focal in 6 and absent in 3. The spleens of 8 animals tested showed a remarkable lack of cells with either θ antigen or surface immunoglobulins. The predominant leukemic cell in these intact animals was thus similar to that seen in immunodepressed mice suggesting the operation of viral as well as host factors.

In summary, LLV induced in thymectomized, ATS-treated mice a leukemia that involved different sites and affected a different subpopulation of lymphocytes from that induced in the intact mouse.

Response Of Cats To Infection With Feline Leukemia Virus: Age-Related Factors And Variation In Disease Caused By Different Virus Strains – ***Edward Hoover, Richard Olsen, William Hardy Jr., Joseph Schaller, Lawrence Mathes & Gary Cockerell, Ohio State Univ.***

To determine the relationship of age to the response of cats to feline leukemia virus (FeLV) infection, 70 specific-pathogen-free cats of various age groups (newborn, 2 weeks, 1 month, 2 months, 3-4 months, and 1 year) were inoculated intraperitoneally with either the Rickard (-R) or the Kawakami-Theilen (-KT) strain of FeLV. To estimate the extent of horizontal transmission of FeLV in each group, age-matched control cats were housed in the same cages with inoculated cats. All cats were monitored continuously for: a) viremia (gs antigen in leukocytes), b) antibody to the feline oncornavirus-associated cell membrane antigen (FOCMA) by immunofluorescence anc cytotoxic assays, c) virus neutralizing (N) antibody, and d) evidence of disease.

Age related variation in susceptibility was found with both FeLV strains. One hundred percent of cats inoculated as newborns and surviving to at least 4 weeks of age developed persistent viremia, produced little or no FOCMA or N antibody, and died of lymphosarcoma, anemia, or other FeLV-related disease. Cats 2 weeks to 2 months of age also appear to be relatively susceptible to infection and oncogenesis by FeLV, although data for these groups is still being collected. By contrast, only 15% of cats inoculated at 3 months of age or older developed persistent viremia or FeLV-related disease. Cats in this group that remained gs antigen-negative after inoculation all produced FOCMA and N antibody and none developed disease after 40 weeks of observation.

Eight of 9 control cats that shared cages with viremic cats developed evidence of horizontal exposure to FeLV (gs antigen or seroconversion to FOCMA and N antibody). Eighteen other control cats housed in the same room, but with non-viremic cage mates, did not develop evidence of FeLV exposure by any test.

The disease induced in susceptible cats varied with virus strain. FeLV-R induced thymic lymphosarcoma in 85% of cats inoculated as newborns. The remaining 15% of the cats died of bacterial infection or thymic atrophy syndrome. FeLV-KT induced fatal non-regenerative anemia associated with erythroid hypoplasia in the marrow and no evidence of neoplasia.

The results of these experiments inducate that: 1) there is substantial age-related variation in susceptibility of cats to FeLV, 2) after exposure of cats to FeLV, the host/virus relationship can be determined and the biologic response predicted by the combination of the immunofluorescence test for FeLV-gs antigen and the immunofluorescence or cytotoxicity tests for FOCMA antibody, 3) efficient horizontal transmis-

sion of FeLV appears to require relatively close contact between cats, and 4) the biologic response of cats to FeLV infection varies with viral strain and includes fatal non-neoplastic as well as neoplastic disease.

Antigenic Markers For Human Leukemia And Lymphoma Cells: Detection With Simian Antisera – ***T. Mohanakumar, R.S. Metzgar & D.S. Miller, Duke Univ. Medical Center***

Nonhuman primate antisera to different morphological classes of human leukemia cells after absorptions with normal buffy coat leukocytes (HWBC) were cytotoxic only to cells from patients with leukemia and some myeloproliferative disorders. These antisera are capable of differentiating between antigens associated with lymphocytic and myeloid leukemias. The initial detailed serological characterization of these antisera have been published (J Nat. Cancer Inst. 52, 1435-1444, 1974). Since then we have produced additional simian antisera, some to cells from acute lymphocytic leukemia (ALL) patients. These antisera after absorptions with HWBC (including T lymphocytes) were cytotoxic to cells from all ALL patients but reacted only with cells from a few chronic lymphocytic (CLL) donors.

Anti-ALL sera after absorptions with HWBC were cytotoxic for normal human thymus cells whereas anti-CLL sera were not. Absorption studies of anti-ALL sera with thymus cells also suggested that the leukemia associated antigenic activity on ALL cells can be distinguished from that on thymus cells. The above finding, along with our earlier published data, suggest that, although certain antigens expressed on human ALL and CLL cells are cross reactive, some membrane associated antigens are unique for cells from ALL donors. The cytotoxic reactivity of the anti-ALL sera to thymus cells also indjcate an antigenic relationship between thymus cells and ALL which is in agreement with the view that in most of these patients the circulating leukemic lymphocytes express certain properties of T lymphocytes.

We earlier reported that cells from lymphoma patients were lysed by rabbit antisera to human leukemia cells but not by simian antisera. Therefore, an antiserum was produced in a monkey to peripheral blood cells from a lymphosarcoma patient at a time when the original disease became leukemic. The antiserum, after absorption with HWBC, was cytotoxic to cells from all lymphoma, lymphosarcoma cells tested. In addition, this antiserum also lysed cells from some leukemia patients irrespective of the morphological classification of their disease. The reactivity of cells from lymphoma patients with monkey anti-lymphoma serum but not with monkey anti-leukemia sera, suggests that lymphoma specific membrane antigens on circulating mononuclear cells of these patients can be distinguished from leukemia associated antigens. The ability of the simian antisera to differentiate between lymphocytic and lyelogenous type of leukemias also appears to be particularly useful in the diagnosis of undifferentiated leukemias.

Human Papovaviruses: Current Knowledge On Their Biologic And Oncogenic Properties – ***Kenneth Takemoto, NIH***

In recent years, two related but serologically distinct classes of hemagglutinating papovaviruses have been isolated from hymans, the prototype viruses being JC virus (JCV) isolated from brain tissue of a case of progressive multifocal leukoencelphalopathy and the BK virus (BKV) isolated from the urine of a renal allograft recipient. Current knowledge on the biology of these viruses is summarized as follows:

1. Prevalence in humans. Both viruses are ubiquitous in the human population; 70 to 80% of adults have serologic evidence of infection.
2. Oncogenicity. BKV and JCV are oncogenic for hamsters, but differ in their ability to produce tumors. JCV is highly oncogenic and produces brain tumors in over 80% of inoculated animals; it also causes tumors in over 50% of hamsters when injected intraperitoneally or subcutaneously. BKV produces fibrosarcomas and ependymomas in a very small percentage of animals, and is only weakly oncogenic.
3. Transformation in vitro. Transformation by BKV or its DNA has been demonstrated in hamster cells.
4. T-antigens. JCV and BKV tumors or transformed cells, or cells lytically infected by these viruses synthesize T-antigens which cross-react with each other as well as with SV40. No antigenic differences between the T-antigens induced by the three viruses have been observed.
5. Antigenic relationship. Besides common T-antigens, JCV, BKV, and SV40 have virion antigens which are distantly related.
6. Extent of homology of DNA's. 10 to 20% polynucleotide sequence homology has been detected in the genomes of the three viruses. Common sequences are all in the "late" regions of the DNA's.

Because of the proven oncogenicity of the new human papovaviruses, their possible role in human neoplasia needs to be assessed. In particular, neoplams involving organs where the virus has been observed (kidney and brain) should be examined by methods known to yield virus or their antigens.

Antilymphocytic Factor Derived From A Herpesvirus Saimiri Lymphoid Tumor Cell Line – ***Russell Neubauer, William Wallen and Harvey Rabin, Litton Bionetics***

Peripheral blood lymphocytes (PBL) from Herpesvirus saimiri (HVS) infected owl monkeys (Aotus trivirgatus) showed a depression in response to general mitogens during the development of lymphoma. This depression was conferrable to PBL of normal monkeys in vitro, inducating the presence of a suppressor

cell population. Present studies show that an HVS-tumor cell line (MLC-1) was capable of inhibiting the mitogenic response of normal PBL, suggesting that tumor cells may be the suppressor cells in vivo. In addition, the mitogenic response was suppressed by both cell-free extracts of MLC-1 cells and their concentrated tissue culture fluids. The extracts inhibited the lymphocyte response to phytohemagglutinin (PHA) at levels as low as 0.24 mg/ml protein and demonstrated anti-HVS activity as determined by plaque inhibition in vero cells to levels of only 100 mg/ml. No effect on vero cell growth or DNA synthesis was noted at levels as high as 400 mg/ml.

Purified human interferon did not inhibit the PHA response of normal PBL at levels as high as 80,000 units/ml. MLC-1 extracts also exerted an inhibitory effect on two out of five established lymphoblastoid cell lines. DNA synthesis appeared to be the most sensitive (95% inhibition) to this factor although RNA and protein synthesis were inhibited but to lesser degrees (60-85%). Inhibition of this type was not noted with interferon (levels as high as 80,000 units/ml). The effect on lymphoblastoid cell lines did not affect cellular respiration and was reversible. These studies demonstrate the presence of a suppressive factor produced by HVS tumor cells which function in vivo to inhibit immunocompetence and therefore may be of importance in the pathogenesis of lymphoma.

Epstein-Barr Virus (EBV)-Specific IgA Antibodies In Nasopharyngeal Carcinoma – ***Gertrude Henle & Werner Henle, Children's Hospital of Philadelphia***

An etiologic role of EBV, the cause of infectious mononucleosis (IM), has become increasingly probable in Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). The spectra and titers of antibodies to EBV-related antigens in these malignancies differ strikingly from those seen in control groups, EBV DNA is detectable in the great majority of biopsies of either tumor, and EBV-associated nuclear antigen (EBNA) is demonstrable in the BL and anaplastic or poorly differentiated NPC cells. While B lymphocytes are readily transformed by EBV in vitro into lymphoblasts with permanent growth potential and malignant attributes, transformation of other cells has not as yet been reported.

The EBV-related serologic patterns in IM, BL and NPC differ not only from those of healthy donors but also among each other. Only in primary EBV infections, such as IM, are high but transient titers of IgM antibodies to EB viral capsid antigen (VCA) observed, absence of anti-EBNA in the presence of VCA-specific IgG antibodies, and transitory antibody responses to the D (diffuse) component of the early antigen (EA) complex. In BL, high titers of antibodies to the R (restricted) component of the EA complex are often noted, which may exceed the generally high VCA-specific IgG titers, and anti-EBNA may range from very high (excessive antigenic stimulation) to very low titers due possibly to defective cell-mediated immunity or antigen-antibody complex formation. In NPC, high titers of anti-VCA as well as anti-D develop with increasing tumor burden from stages I to V which gradually decline again to lower (anti-VCA) or non-detectable levels (anti-D) following effective therapy. Anti-EBNA titers are usually high in this malignancy.

Another outstanding serologic feature of NPC has come to light recently. Nearly all patients show prior to treatment serum IgA antibodies to VCA and frequently also to D, often at substantial titers which may equal the corresponding IgG antibody levels. The IgA antibody titers increase with tumor burden and both VCA- and D-specific IgA may disappear after effective therapy. In contrast, only 2% of patients with other carcinomas or healthy donors and less than 30% of BL patients have VCA-specific IgA antibodies, mostly at low titers, and no more than 40% of IM patients show transient, weak VCA-specific, rarely D-specific IgA responses. The incidence and titers of IgA antibodies to herpes simplex type 1 virus are not elevated in NPC and comparable to those seen in other carcinomas or healthy controls. It remains to be determined whether EBV-specific IgA antibodies in NPC are of the secretory (11S) or non-secretory (7S) type which should provide clues to the site of their origin and their unusual prevalence in this tumor.

Chemoimmunotherapy In Human And Experimental Leukemia – ***J. George Bekesi, Julia Roboz, & James Holland, Mount Sinai School of Medicine***

The AKR strain of mice are destined genetically to develop virus induced lymphatic leukemia. By 12 months of age about 95% of AKR mice die of leukemia. At the time of diagnosis there are 0.6 to 1.8 x 10^9 widely disseminated lymphoma cells in AKR mice. If no cytoreductive therapy is used, the AKR mice die after diagnosis of spontaneous leukemia: 50% by 14 days and 90% by 33 days. Leukemic AKR mice treated with vincristine + palmO-ara-C or cytoxan followed by methyl CCNU sustained an increase of lifespan about 180% but less than 5% of animals survived beyond 100 days. Combination chemotherapy + immunization with neuraminidase-treated spontaneous leukemic thymocytes or with allogeneic E_2G leukemic cells intradermally resulted in 25-35% of animals surviving beyond 150 days without evidence of the disease. It is particularly significant that the allogeneic (Gross virus induced) E_2G leukemic cells used as immunogen in animals brought into remission with combination chemotherapy was as effective in prolonging the lifespan of the injected leukemic host as the syngeneic leukemic thymocytes.

The experimental data led to clinical trial in acute myelocytic leukemia using chemotherapy combined with neuraminidase-treated allogeneic myeloblasts. Patients were allocated to two groups following successful remission induction using cytosine arabino-

side and daunorubicin. All received cyclical maintenance chemotherapy every four weeks. The group randomized to receive immunotherapy in addition was treated with Neuraminidase-treated allogeneic myeloblasts intradermally monthly. At each immunization, 10^{10} neuraminidase-treated cells were injected in approximately 40 sites in different lymph node drainage areas. Of 10 patients who received previous antileukemic therapy, six immunized patients had more than twice the remission duration of the four controls. Of 18 previously untreated patients, the median remission duration on chemotherapy alone was 22 weeks for nine patients, while six of nine patients receiving chemo-immunotherapy remain in remission from 68 in 115 weeks. Immunized patients show greater evidence of cellular immune response in vitro and in vivo than the control patients.

Intracellular Forms Of Epstein-Barr Virus DNA In Latency – ***A. Adams, T. Lindahl, Ch. Dierich, G. Bornkamm & G. Bjursell, Karolinska Institute, Stockholm***

Many human lymphoid cell lines contain several copies of the Epstein-Barr virus (EBV) genome without being virus producers. In such cell,s the virus DNA is present in two different forms: as non-integrated viral genomes, and as integrated DNA. The non-integrated EBV DNA has a covalently closed circular conformation, according to centrifugation analysis in neutral and alkaline glycerol gradients and in ethidium bromide/CsCl gradients. In purified fractions of such DNA, circular superhelical DNA molecules of the size of the EBV genome have also been visualized by electron microscopy. Corresponding DNA fractions from EBV-negative human lymphoid cell lines (Molt-4, BJA-B) do not contain such circular DNA molecules.

In addition to this non-integrated intracellular form of EBV DNA, viral DNA sequences with properties of integrated DNA have been isolated by repeated banding in CsCl gradients. Both non-integrated circular forms and integrated sequences of EBV DNA have also been found in tumor biopsies from Burkitt lymphoma patients, and in nasopharyngeal carcinoma tumor cells grown in nude mice. The data indicate that EBV has the properties of an episome during latency.

CONTRACT AWARDS

Title: Improvement in migration inhibition assay
Contractor: Univ. of Texas Health Science Center (San Antonio), $75,609.

Title: Preparation and purification of viral components
Contractor: Pfizer, Inc., $149,806.

Title: Hematology support care project
Contractor: Microbiological Associates, $300,000.

NCI ADVISORY GROUP, OTHER CANCER MEETINGS SCHEDULED FOR NOVEMBER

Virus Cancer Program Scientific Review Committee A—Nov. 2, Hershey (Pa.) Motor Lodge, open 2–2:30 p.m.
Virus Cancer Program Scientific Review Committee B—Nov. 2, Hershey Motor Lodge, open 9–9:30 a.m.
Tenth Annual Joint Working Conference of the Virus Cancer Program—Nov. 3–5, Hershey Motor Lodge, 9 a.m.–5:30 p.m. Nov. 3 & 4; 9 a.m.–noon Nov. 5, all open.
Combined Committees of the Breast Cancer Task Force—Nov. 5, NIH Bldg 31 Room 6, 8:30 a.m.–5 p.m., all open.
Breast Cancer Experimental Biology Committee—Nov. 6, NIH Bldg 31 Room 5, open 8:30–10 a.m.
Breast Cancer Treatment Committee—Nov. 6, NIH Bldg 31 Room 10, open 8:30–10 a.m.
Breast Cancer Epidemiology Committee—Nov. 6, NIH Bldg 31 Room 7, open 9–11 a.m.
Drug Development Committee—Nov. 7, Blair Bldg Room 414, open 9–9:15 a.m.
Board of Scientific Counselors, Div. of Cancer Treatment—Nov. 10-11, NIH Bldg 31 Room 8, 9 a.m. both days, all open.
NCAB Subcommittee on Environmental Carcinogenesis—Nov. 10-11, NIH Bldg 37 Room 1B04, 9 a.m. both days, all open.
NCAB Subcommittee on Centers—Nov. 16, NIH Bldg 31 Room 8, open 7:30–9 p.m.
National Cancer Advisory Board—Nov. 17-18, NIH Bldg 31 Room 6. Open Nov. 17 9–9:15 a.m., Nov. 18 9 a.m.–noon.
Carcinogenesis Program Scientific Review Committee A—Nov. 19, Landow Bldg Room B301, open 9–9:30 a.m.
Developmental Therapeutics Committee—Nov. 19, NIH Bldg 37 Room 6B23, open 8:30–9:30 a.m.
Seminars in Cancers Involving the Skin—Nov. 19, Roswell Park, 9 a.m.–5 p.m., registration required.
Retinoids and Cancer Prevention Workshop—Nov. 20-21, NIH Bldg 31 Room 4, 8:45a.m.–5 p.m., all open.
Committee on Cancer Immunotherapy—Nov. 20, NIH Bldg 10, Room 4B14, open 1–1:30 p.m.
Combined Modality Committee—Nov. 25, NIH Bldg 31 Room 10, open 8:30–9 a.m.

SOLE SOURCE NEGOTIATIONS

Proposals are listed here for information purposes only. RFPs are not available.

Title: Studies on isolation and characterization of type C viruses and diagnostic testing and service functions
Contractor: Microbiological Associates.

Title: Studies on viral-chemical carcinogenesis
Contractor: Microbiological Associates.

Title: Continuation population-based cancer epidemiology research center in Iowa
Contractor: Univ. of Iowa.

Title: Support services for the application of animal virus model system to human neoplasia
Contractor: Litton Bionetics.

The Cancer Newsletter—*Editor* JERRY D. BOYD

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