Discussion Following Papers by Drs. Rubin and Heidelberger

Prepared by D. Bernard Amos, Chairman

Dr. Eva Klein said: "In our laboratory, H.O. Sjögren, K. E. Hellström, and G. Klein studied the transplantation behavior of polyoma-induced tumors. Tumors of different histological types were induced and transplanted in inbred mouse strains; they include fibrosarcomas, undifferentiated sarcomas, osteogenic sarcoma, thymoma and epithelial tumors.

"From 32 tumors, 35 could be transplanted into mice belonging to the same strain. All tumors used in the experiments which I am going to report were studied in their very early transplant generations.

"Tumor cells were transplanted to untreated isologous mice and to mice pretreated 4 times with tissue culture fluid, containing polyoma virus. The virus was derived from mouse embryo monolayer cultures infected with polyoma virus 14 days earlier. Such tissue culture supernatants contained virus in a hemagglutination titer of from 1 to 250, up to 1 in 1,000. Mice given injections of this virus preparation developed high titers of antiviral (HI) antibodies. In some experiments purified virus preparation was used for pretreatment, obtained by absorption to, and elution from, guinea pig red cells.

"In summary of the results obtained with sixteen polyoma-induced tumors, 87 per cent (161/184) of the transplantations were successful in the untreated isologous recipients. Only 28 per cent (53/189) of the mice given injections of purified virus or infected tissue culture supernatant supported the tumor. The virus-pretreated mice, in which the tumor grew often, showed a longer latency period and a delayed growth as compared with the untreated controls.

"Five mammary tumors and three methylcholanthrene-induced sarcomas obtained from the polyoma-free colony were tested in the same way. Their transplantation behavior was not influenced by pretreatment of the recipient mice with polyoma virus, and they grew just as well as in untreated controls.

"Mice infected when newborn with polyoma virus and containing high titers of antiviral antibodies also showed resistance to the transplantation of the established polyoma tumors. Untreated mice, given inoculations of low cell doses, developed tumors regularly, but mice given injections of polyoma virus when newborn developed no or only a few tumors.

"The mechanism of these results is unclear at present. It is not known whether the difference between untreated and virus-immunized mice is due to the effect of the antiviral antibodies on the tumor cells, or, alternatively, to the presence of a new cellular antigen in polyoma-infected cells. An antigen of the latter type might be speculated to appear, e.g., in analogy with lysogenic conversion in bacteria, in those cells that survived virus infection and carry the virus in pro-virus form or in some masked form. Experiments are being carried out presently by Sjögren in our laboratory to distinguish between the possible effect of antiviral antibodies and a cellular antigen of the type mentioned."

Dr. Bogden discussed the production of abnormal globulins in multiple myeloma and then presented results of his analysis of sera from inbred P. A. rats bearing methylcholanthrene-induced tumors. Sera were obtained from the normal animal, through the induction phase, during tumor growth, after tumor excision, and after immunizing procedures. They were examined for glycoproteins and total proteins by paper-strip electrophoresis with the use of Tris-EDTA-boric acid (TEB) buffer and veronal buffer.

"The serum of different animals all bearing methylcholanthrene-induced primary tumors of a similar histologic type produced characteristic and differentiating electrophoretic patterns. The electrophoretic pattern was detectable most clearly in TEB buffer, and the differentiating component was a glycoprotein with alpha-2 globulin mobility."

"The alpha-2 globulin glycoprotein detected in TEB buffer is not the alpha-2 globulin which numerous investigators working with barbiturate buffers have reported to be increased in malignancies. The two patterns obtained with the same samples of serum emphasize the dependence of the electrophoretic pattern on the buffer.

"The glycoprotein which we have found to be
characteristic of certain primary tumor-bearing sera appears as a “new” band in the alpha-2 region of TEB, whereas this component appears to be masked in the classic five electrophoretic regions produced in veronal. Furthermore, results of electrophoretic studies of the changes in the globulin glycoproteins under various conditions of “rapid growth” (unpublished data) indicate that an increase of the TEB alpha-3 component characterizes rapid growth whether the tissue is normal (isologous embryonic tissue implants, after partial heptatectomy) or malignant (primary and transplantable tumors).

“The stability and heritability of the electrophoretic pattern are indicated by its reappearance in the serum of littermates bearing isografts, its prompt disappearance following surgical extirpation, and its reappearance following progressive growth of autografts. These latter results would also indicate that, as with the plasma-cell neoplasms or the leukemias, the glyco-protein with alpha-2 globulin mobility which we have detected in the sera of certain primary tumor-bearing rats may also be a cellular constituent originating in the tumor.

“Although a greatly increased production of the globulins has been found to be characteristic of neoplasms originating from reticulo-endothelial tissues, our findings of a glycoprotein peculiar to a number of fibrosarcomas would indicate the possibility that abnormal protein synthesis, detectable electrophoretically, is not restricted to neoplasms originating from the classical globulin-producing tissues. The fact that the peculiar alpha-2 glycoprotein component was not found in the serum of all rats bearing histologically similar sarcomas indicates that all such sarcomas are not alike. There is also the possibility that an anomalous glycoprotein of different electrophoretic mobility is being masked in the alpha-1 and alpha-3 globulin regions in those rats whose serum did not show the characteristic alpha-2 peak in TEB, since both the alpha-1 and alpha-3 globulin components are increased in these animals.

“A rationale for the differences in the proteins synthesized by histologically similar sarcomas is indicated by Potter and Fahey1 who postulate that the individuality of myeloma-globulin synthesis, for example, may stem from a specialization established in normal plasma cells before carcinogenesis. The plasma-cell population being composed of multiple fixed cell types or clones, carcinogenesis begins in and involves only one of these clones.

“... indicates by Potter and Fahey1 who postulate that the individuality of myeloma-globulin synthesis, for example, may stem from a specialization established in normal plasma cells before carcinogenesis. The division of labor, as visualized with antibody-forming cells, would in neoplasms be related to differences in the electrophoretic mobility of globulins or other proteins entering body fluids. Our ability to detect these substances electrophoretically is not only dependent upon their concentration but, as our studies have shown, also upon the buffer used for their fractionation.

“Following through with Doctor Rapport’s second premise in the search for ‘cancer specific antigens,’ an immunologic proof of ‘cancer specificity’ would not be required for such easily detectable changes in the protein constituents of the blood when applied to the evaluation of response to therapeutic procedures, or their possible use as a prognostic tool.

“However, a rather interesting question presents itself: If proteins originating from neoplasms and found in the humoral fluids have an antigenic individuality related to the neoplastic process, what role may they play in the stimulation or neutralization of the host’s response to autochthonous cancer?”

DR. SACKS reported that “Acute infection with a filtrable agent of rats has been demonstrated to produce a syndrome of acute intravascular hemolytic anemia (the agent has been abbreviated, FHA, for Filterable Hemolytic Anemia). Recovery from infection in rats is associated with the development of immunity to the agent itself and resistance to transplantable tumors.3, 4, 5 Evidence has been accumulated to support the view that the mechanism for the resistance to transplantable tumors is at least partially on an immunological basis: Adult rats recovered from infection with FHA possess antibodies directed against the FHA agent and have proved resistant to the transplantation of standard ordinarily easily transplantable rat tumors (six tumors have been tested); sera from rats convalescent from infection with FHA produce cytotoxic changes in living, intact tumor cells but produce no apparent effect on normal, non-

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malignant cells; and, lastly, sera from rats recovered from FHA infection neutralize the viability and transplantability of Flexner-Jobling rat carcinoma cells in vitro. It is possible that the tumor immunity induced by infection with FHA is due to the production of antibodies to antigens shared by the FHA agent and transplantable rat tumors but absent from normal cells. Newborn ratlings respond quite differently to inoculation with the FHA agent. Ratlings administered FHA agent during the newborn period do not develop any detectable disease syndrome. There is no lethality associated with infection by FHA in the neonatal period. Readministration of FHA agent to adult rats previously inoculated with FHA during the newborn period does not result in the production of an acute hemolytic anemia syndrome. This discussion is a presentation of studies designed to determine the effect of inoculation of FHA during the newborn period on susceptibility to tumor transplantation during adult life, and also the effect of this inoculation on the ability to develop FHA tumor immunity later in life.

"Whereas recovery from FHA infection in rats beyond the neonatal period is associated with the development of tumor immunity, administration of FHA to ratlings within 24 hours of birth not only induces no resistance to tumors but renders the animals incapable of subsequently developing FHA tumor immunity. Several explanations can be offered to clarify the apparent inability to produce tumor immunity in newborn animals treated with FHA. First, it is possible that newborn ratlings administered FHA with or without subsequent doses of FHA during their life span received, in fact, an inadequate antigenic stimulation by the FHA agent. The lack of development of FHA tumor immunity in newborn ratlings treated with FHA with or without subsequent reinoculation of FHA during later life could be explained by the very markedly limited antibody responsiveness of the newborn animal and on the basis of a quantity of nonreplicating antigen insufficient to provoke an antibody response. While this suggestion cannot be refuted, it seems unlikely. It does not explain the results with the Novikoff hepatoma which tend to indicate that transplantation of this tumor is more virulent for rats given FHA in the newborn period than for control normal animals or ratlings administered normal blood within 24 hours after birth.

"A second possibility assumes that FHA tumor immunity is produced by auto-immunization against cellular breakdown products released during the course of infection with FHA. The newborn animals inoculated with FHA, failing to develop tissue destruction in response to either the first or subsequent administrations of FHA, would not be exposed to a sudden release of cellular components, and therefore, could not develop an auto-immunity. However, there is no evidence that FHA infection results in an auto-immunity. In fact, serum from FHA-recovered animals has been found to be ineffective in inducing cytotoxic changes in normal rat cells. This viewpoint also fails to explain the somewhat greater mortality and decreased survival time of FHA newborns in comparison to normal newborns transplanted with the Novikoff hepatoma.

The last hypothesis, and the one the author favors, is that inoculation of newborn ratlings with FHA results in an acquired immunological tolerance to the FHA agent itself and therefore renders the animals incapable of forming antibodies against FHA and developing FHA tumor immunity. This hypothesis includes as a corollary a shared common antigenicity between the FHA agent and rat tumor cells (this has been discussed elsewhere). In one experiment, FHA newborns had the shortest survival time and the greatest mortality following transplantation of the Novikoff hepatoma. This group was followed by the normal newborns and by the normals. Statistically significant differences in survival time were found only between the first and latter groups, however. Nevertheless, it would appear that these three groups are different from one another. The greater virulence of tumor transplantation for newborn animals administered normal blood in comparison to normal animals can be explained by another phenomenon. The tumor employed is not strain-specific, and therefore a certain degree of genetic discrepancy exists between the tumor and host. The administration of homologous rat blood to newborns is capable of inducing an acquired tolerance to the administered cellular antigens. Normal animals would be theoretically capable of reacting against homotransplant antigens in the transplanted tumor, whereas the animals administered homologous rat blood during the newborn period, having an acquired tolerance to some rat homologous antigens, would be incapable of responding immunologically to those antigens to which an acquired tolerance was produced. It is not surprising, therefore, to expect a certain increase of virulence of the tumor transplant in this latter group. An additional increment of virulence of the transplanted tumor for the FHA-treated newborns is found. Presumably both the normal newborns and the normals, while having no preformed immunity, are still capable of responding, at least
to a certain degree, to the antigen(s) shared by the FHA agent and tumor cells, whereas the FHA tolerant animals are not. FHA tumor immunity, of course, cannot be equated with homologous transplant immunity because of the ease of development of FHA tumor immunity by the administration of FHA during adult life to animals previously treated in the newborn period with normal rat blood. The serious shortcoming of this explanation is that it does not account for the apparent inability of the FHA agent to induce a recognizable syndrome in the newborn rat. Also, it fails to explain the identical virulence of the Miller hepatoma for FHA newborns and normals.

"The explanations offered of the absence of FHA tumor immunity in rats given inoculations during neonatal life of FHA and the mechanisms involved in the inability of such animals to develop this tumor immunity later in life are totally speculative. Studies are presently in progress in attempts to demonstrate and, if possible, define any common antigens of the FHA agent and rat tumor cells, determine the fate and infectivity of the FHA agent in the newborn rat, and define directly the mechanism of 'acquired tolerance' to FHA tumor immunity."

Dr. M. C. Niu reported that the tumor-producing capacity of Nelson ascites tumor cells decreased significantly after treatment with liver RNA (Science, 131:1821, 1960) but not with RNA from tumors. Most of the treated cells do not take up eosin, and 10 per cent of animals given injections do develop tumors, although there are indications of reduced mitotic activity and invasiveness and of the development of structural organization.

"The incorporation of amino acid-C\textsuperscript{14} into proteins is impaired by the presence of 2.4 mg liver RNA/ml but not by 0.6 mg/ml. Assay was made of protein precipitated by trichloroacetic acid, extracted with alcohol, dialyzed, and then precipitated with antiserum against serum albumin. Synthesis of this protein increases during the incubation of the tumor cells with liver RNA. After 3 weeks' incubation the concentration of serum albumin-like protein was proportional to the concentration of liver RNA. Dr. Niu believed that contamination of the liver RNA with serum albumin was unlikely, because "cells treated with either serum albumin alone or tumor RNA plus albumin do not create an increase" (although exchange of labeled amino acid could occur).

Dr. Niu concluded that ascites cells treated with liver RNA lose tumor-forming potential and become capable of synthesizing a serum albumin-like protein specific to the tissue source of RNA."
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