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Summary: Antigens of Chemically Induced Tumors; and Search for Tumor-specific Antigens in Other Human Cancers

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After Dr. Prehn's lecture, the question arose, in relation to the theory of immunoselection proposed by him, whether there was any demonstrable relationship between lymphocyte infiltration and the appearance of normal (revertant?) mammary gland tissue in serially transplanted hyperplastic nodules. Dr. Prehn made it clear that lymphocytic infiltration was visible in the areas that remained hyperplastic, whereas there was little or no infiltration in the areas where the outgrowth appeared normal. This was to be expected if the normal tissue was nonantigenic. Dr. Old mentioned that the work of the Sloan-Kettering group on methylcholanthrene-induced guinea pig tumors showed that soluble tumor materials were immunogenic in delayed hypersensitivity and tumor rejection tests as well. Both tests revealed similar patterns of individually distinct antigenicity in different sarcomas. In reference to the frequently demonstrated individual antigenicity of chemically induced tumors, Dr. Day brought up the question of whether the theory of latent virus activation by carcinogen action, based *inter alia* on the experiments of Duran-Reynals and Bryan (Ann. N. Y. Acad. Sci., *54:* 977–991, 1952), could be ruled out as an important mechanism in chemical carcinogenesis since wider cross-reactions might be expected in that case. Referring particularly to the work of Dr. Old, Dr. Prehn replied that common antigens determined by vertically transmitted viruses would not be immunogenic due to host tolerance, and that this is now widely demonstrated in numerous systems. In the case of the mammary tumor in mammary tumor virus-bearing C3H mice where tolerance is known to obscure a common antigen in this fashion, Dr. Blair has recently demonstrated the existence of superimposed weak and individually different antigenic specificities in different carcinomas. For this reason, Dr. Prehn did not feel that individual antigens necessarily exclude an underlying common specificity.

Answering a question from Dr. Dent, Dr. Prehn went on to discuss immunoselection in established, methylcholanthrene-induced tumors. He pointed out that antigenic tumors tend to throw off less antigenic variants on serial transplantation and attributed this to immunoselection, partly because of the variability found by him with respect to the immunosensitivity of different subpopulations selected from the same original tumor, and partly because the loss in immunosensitivity was less likely to occur if the tumor was passaged in immunodepressed hosts.

There was considerable discussion around the question whether tumor-specific antigens can be released by living cells or are only liberated in connection with cell degeneration and tissue necrosis. Another related point was whether immunogenicity was dependent upon the integrity of the cell membrane or whether it could also be obtained with soluble fractions, as in the case of the guinea pig systems mentioned by Dr. Old and unlike the usual experience with murine or rat neoplasms of corresponding type. The question of local *versus* systemic sensitization came up as well. Since no conclusive answers could be given to any of these questions, this discussion is not recapitulated in detail.

The second general discussion took place following the papers by Drs. Gold, Abelev, Day, and Kunkel. In a comment on Dr. Gold's paper, Dr. Abelev mentioned the work of Zilber and Liudogovskaya. Using the agar precipitation technic, they demonstrated three antigens in human gastric cancer, absent from normal gastric mucosa. Dr. Abelev demonstrated two of these antigens in the colon mucosa and Avenirova and Liudogovskaya went on to show that the third one is present in small amounts in the spleen. They could not demonstrate any extra antigens absent from the colon mucosa and present in gastric cancer. Up to now, they did not find antigens of the carcinoembryonic type in gastric tumors. Dr. Gold felt that these findings were similar to those reported by Burtin *et al.* on gastric carcinoma. Gold has found that cancers localized at different levels of the gastrointestinal tract contained different concentrations of the carcinoembryonic antigens (CEA) of the human digestive system. The higher the tumor, the lower the CEA concentrations. At the level of the stomach the concentration was quite low. Gold also said that his immunizations and initial tests were carried out with large bowel tumors in which the CEA concentration was relatively high and the tumor-specific antigen-antibody system was readily demonstrable. Similar antigens were then found in gastric cancers, first by precipitin inhibition and only later by direct Ouchterlony reactions, using high concentrations of gastric cancer extracts. Thus, in experiments where initial immunizations have been performed with gastric tumor extracts, it is not unlikely that a relatively weak anti-CEA response might have been obtained. Dr. Gold felt that this possibility, together with testing against relatively low concentrations of gastric cancer tissue extracts, could have led to the CEA-anti-CEA system being overlooked.

Responding to this discussion, Dr. Ovary reported some experiments with human colon carcinoma that were performed at New York University Medical School by Dr. Kronman; he

found that human colon and human colon carcinoma are antigenic in rabbits. Using the double diffusion technic of Ouchterlony, it was seen that rabbits produce antibodies against several antigenic determinants either against normal human colon or against human colon carcinoma as antigens. When antiserum against normal human colon was absorbed with either normal human colon or by human colon carcinoma, no precipitin lines could be produced either against normal human colon or against human carcinoma. However, when antiserum against human colon carcinoma was absorbed with normal human colon, one precipitin line could still be seen against human colon carcinoma, but not against normal human colon. Absorption by human colon carcinoma prevented the formation of any precipitin line in all antisera. The interesting finding is that each rabbit was immunized with specimens of normal human colon or human colon carcinoma coming from different cases, and, nevertheless, each antiserum could be absorbed with any of the colon carcinoma specimens. This fact already showed the identity of some antigenic determinants present in all cases of human colon carcinoma examined. Moreover, with the double diffusion technic of Ouchterlony, an absolute identity between the precipitin lines specific for the colon carcinoma was seen. Dr. Ovary felt that these results were quite similar to those of Dr. Gold with respect to the antigen in embryonic colon, particularly in view of the individual immunization and the identity of the antigen in all cases studied.

Dr. Milgrom asked Dr. Gold whether the CEA activity demonstrated by him could be related to the "necrotic tissue antigens" found by Hirszfeld *et al.* in various tumors. Dr. Gold replied that he had studied a number of noncancerous necrotic tissues derived from the digestive system or from other tissues, and none of these showed the presence of the CEA characteristics of the human digestive system. When asked to clarify the methods used in demonstrating the anti-CEA antibodies, Dr. Gold said that the rabbit antisera were tested by precipitation in agar gel, bis-diazotized benzidine (BDB)-hemagglutination, immunofluorescence, and by passive cutaneous anaphylaxis in mice. Human sera were studied only by BDB-hemagglutination, whereas precipitin tests were negative. Dr. Gold felt that this could be expected in view of the relatively low titers obtained in the BDB-hemagglutination tests.

After listening to Dr. Gold, Dr. Dent wished to make a more general comment on the role of humoral antibodies in tumor resistance. He felt that there was little evidence to suggest that the presence of humoral antibodies was a good prognostic sign. Referring to the theories of Thomas (*In:* H. S. Lawrence (ed.), Cellular and Humoral Aspects of the Hypersensitive States. New York: P. B. Hoeber, 1959) and of Burnet (Science, *133*: 307–311, 1961), he stressed the importance of cell-mediated reactions. He felt that there was much evidence to suggest that cellular immunity is more important in resistance to tumor antigens and to intracellular or cell-associated microorganisms, such as viruses and mycobacteria. In at least one situation, antibodies would appear to be of little value in eliminating virus from the host. In the congenital rubella syndrome, persistence of virus is associated with a high IgM antibody titer. In two instances which have been studied, disappearance of the virus has been associated with a fall in IgM levels and a rise in IgG levels. Whether the qualitative change in antibody class is cause or effect in relation to the termination of virus excretion is difficult to say in terms of our present state of knowledge. In many other systems the conversion of IgM to IgG antibody is a sequential event and may be a reflection of antigen clearance. In regard to Dr. Gold's report of a rising antibody titer in patients whose colon cancer had been removed, Dr. Dent asked whether there was also a change from the IgM to the IgG type of antibody. Dr. Gold had only preliminary evidence on this point, but so far it has shown that the anti-CEA antibody remained mercaptoethanol-sensitive in the sera of patients following resection. There was no evidence concerning the question whether CEA antigens could induce host rejection responses or not.

Responding to other questions concerning the possible role of derepression, perhaps as a result of genetic losses, in determining the reversion of adult antigenic patterns to fetal forms, Dr. Gold mentioned that, although a group of bronchogenic carcinomas showed no evidence of CEA activity, one particular specimen of an anaplastic bronchogenic cancer did show some CEA activity after strong extract concentration.

Several questions were raised concerning the relationship between the antigen described by Dr. Gold and by Dr. Burtin's group in Paris. Dr. Gold stated that reagents were exchanged between the two groups, but both the immunologic and the biochemical analysis indicated that the CEA are different from the "supplementary tumor antigens" described by the Burtin group.

Dr. Porter asked Dr. Abelev whether the α_F globulin, described by him, ever appeared in the serum of a pregnant animal or human female. Dr. Abelev said that α_F *could* be detected in the sera of pregnant mice and rats and probably also in humans. The concentration was very high in the fetal sera but only very low levels could be demonstrated in the blood of the mother. Dr. Abelev felt that the α_F globulin could cross placental barriers, but to a very slight degree.

Concerning the myelomas, Dr. Hellman raised the question whether there was any relationship between the clinical response of the patients to treatment and the class of immunoglobulin they were producing. Dr. Fahey replied that the possible relation of heavy chain class (gamma or alpha) and light chain type (kappa or lambda) to clinical manifestation of multiple myeloma has been investigated by Carbone *et al.* (Am. J. Med., *42:* 937–945, 1967). They found that these factors did not influence survival or response to therapy. Bergsagel found evidence indicating a chemotherapy response difference in a comparison of patients with kappa or lambda Bence Jones proteinuria, but according to Dr. Fahey, this has not been confirmed in other studies. Dr. Fahey did not believe that there were any published investigations of the relationships between clinical features of disease and heavy chain subclass.

Dr. Merrill asked Dr. Kunkel whether the antibody activity demonstrated for certain myeloma products could be explained as being due to antibodies induced in the host by exposure to specific antigens in parallel with the oncogenic process, or

whether it had to be regarded as the reflection of the random transformation of various cell clones. Dr. Kunkel replied that there was no answer to this question as yet. The most interesting case was the patient with the anti-dinitrophenyl myeloma. In this case, there was no evidence that this patient had been exposed to dinitrophenyl, but this was, of course, difficult to rule out conclusively. In the future, before this question can be answered meaningfully, it will be most important to look for antibodies against materials to which the individual has not been exposed.

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