A Short Review of Immunological Investigations on Cancer*

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For an immunologist it seems logical to summarize the extensive investigations performed on cancer immunology under three major headings dealing with hetero-, iso-, and auto-immune reactions.1

HETEROIMMUNE RESPONSE TO TUMOR ANTIGENS

Heteroimmune sera as reagents detecting tumor antigens.—Only those investigators who have spent years in using sera obtained by heteroimmunization for investigations on tissue specificity realize all the drawbacks of this method. Animals immunized with a tissue of foreign species origin respond to many antigens. Several antibodies are directed against serum proteins; some of them may react with organ-specific antigens, and antibodies may be found against blood group antigens and against Forssman and Wassermann antigens. In addition, the pattern of reaction differs considerably in individual antibody-producing animals. It is not my intention to say that heteroimmune sera should not be used as reagents in the investigation of tumor specificity. It is still encouraging that many such antisera have been used successfully for the tracing of organ-specific antigens; and, too, they permitted the discovery of at least two human blood group systems. I want only to emphasize that it certainly requires much experience and skill not to get lost in the jungle of serological reactions produced by heteroimmune sera.

In the late 1920's and early 1930's pioneering serological work on the antigenic structure of human malignant tumors was independently performed by Witebsky and his colleagues (56, 58, 59) in Germany and by Hirszfeld and his collaborators (27, 28) in Poland. Both groups used rabbit immune sera as reagents and the complement fixation test as the main laboratory procedure. These investigations indicated the possible existence of cancer-specific, ethanol-soluble antigens. The possibility of misleading results due to blood group antigens was fully realized. Witebsky apparently obtained specific reactions with extracts of Jensen rat sarcoma in addition to those from human tumors. Subsequently, Hirszfeld and Halber (24) showed that malignant tumors contain antigens similar to the antigens of caseous tuberculous tissue, pus, and infarct tissue. The authors coined the term "necrotic antigens" to denote antigens appearing in various pathological processes and devoid of the specificity for a particular disease. The concept of necrotic antigens was later reinvestigated in connection with the work on immune response to heterografts (34).

Rapport and his collaborators (19, 42-44) have been using rabbit immune sera to investigate, by means of the complement fixation test, the non-protein components of human and animal malignant tissues. These investigations, performed with elegant immunochemical technic and evaluated with objectivity, did not prove the existence of strictly cancer-specific antigens, although they brought evidence that lipid constituents of cancer tissue may differ quantitatively from those of normal tissue.

Björklund and his collaborators (5-7) showed that antisera of horses immunized with pooled human cancer tissue exhibited cytopathogenic activity against the tissue culture of human malignant cells. The cytopathogenic activity could be absorbed by malignant but not by normal

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1 In speaking about cancer immunology we have in mind immune responses to antigens resulting from the transformation of normal tissue components by the pathological process, and we will exclude from our discussion experiments performed on animal tumors of established virus etiology and dealing with immune reactions to viral antigens.
human tissue. This work was recently criticized by Goldstein and Hiramoto (15). To my knowledge, Dr. Björklund never tested sera of horses immunized with normal human tissues for cytotoxic activity. Certainly such a control would seem desirable.

In contrast to the data presented above there were also experiments suggesting the disappearance of some antigens in malignant tissues. Weiler (54, 55) showed that carcinomas of rat liver and hamster kidney do not contain the organ-specific antigens present in the corresponding normal tissues. Seligmann et al. (47) and Milgrom et al. (35) found that leukoblasts of lymphocytic leukemia may be poorer antigenetically than normal leukocytes.

Sensitized animals as "reagents" detecting tumor antigens.—The evaluation of heteroimmunization results is difficult enough when well established serological tests like complement fixation, precipitation, and agglutination are employed; however, it becomes almost a Herculean task when nonorthodox tests such as systemic anaphylaxis and the Schultz-Dale reaction are used.

For about 15 years, Zilber and his school (for references see 53, 62–64) have been performing extensive experimentation on the antigenic composition of malignant tissues of animal and human origin. The main technic employed by Zilber is the active anaphylactic reaction. Guinea pigs are sensitized by the nucleoprotein fraction of tumor extracts and desensitized by a similar fraction prepared from the corresponding normal tissues. Finally, they are challenged intravenously with the tumor nucleoprotein. Anaphylactic response to the challenge injection is considered as a positive result. Zilber and his collaborators claimed to prove the presence of specific antigens in human carcinoma, sarcoma, and leukoses, as well as in spontaneous and cancerogen-induced animal tumors.

The experiments of Makari and his collaborators (33) were based on a similar principle as Zilber's experiments. The Schultz-Dale technic with isolated uterine horns of sensitized guinea pigs was employed instead of systemic anaphylaxis. Desensitization was performed with normal serum and the challenge with cancer serum. The usefulness of Makari's technic was confirmed by three groups of investigators (8, 18, 22) even if their results were not always as encouraging as the original findings.

The work accomplished by Zilber and his collaborators, as well as the interesting technic of Makari and his followers, is to be greatly admired. However, one still wonders whether the exciting results obtained by these investigators were not influenced by the antigenic complexity of tissues. Not only the blood group antigens (certainly only some of them could be kept under control) but also serum group antigens and other iso-antigens could influence some experimental results. Further experimental data obtained with these technics are eagerly awaited.

Heteroimmune sera in tumor therapy.—On the basis of experience with the in vivo effects of heteroimmune sera against normal organs, one would not consider serotherapy of solid tumors as promising at the present time. Whereas heteroimmune sera easily destroy circulating cells (experimental anemia, leukopenia, and thrombocytopenia), selective organ destruction has not been accomplished by injecting such sera. The only exception might be the Masugi type of nephritis (for reference see 46); however, the pathogenesis of this peculiar experimental disease is far from being clarified, and final evidence that kidney-specific antibody is active in this disease has still to be elaborated. In addition, crude anticancer sera containing antibodies against normal tissue antigens may induce more harm than benefit, to say nothing about possible serum sickness and other allergic manifestations.

This situation certainly may be changed when sera purified by selective absorption and chemical procedures are employed. Extensive experimentation on localization of labeled antibodies and on selective absorption of antisera has been performed by Pressman and his collaborators (39–41). Possibly this type of investigation will give the final answer about serotherapy of cancer.

Resistance to tumors of foreign species.—In his pioneering experiments performed more than half a century ago, Ehrlich (11) found that a mouse tumor transplanted to a rat does not "take" but is rejected in a few days. Ehrlich attempted to explain this finding by "athreptic immunity": the rat lacks a certain nourishing substance, X, which is necessary for the growth of a mouse tumor. Now it is obvious that the rejection of foreign species tumors is due to the immune answer to heteroantigens as is the rejection of any heterograft.

ISOIMMUNE RESPONSE TO TUMOR ANTIGENS

The prefix iso as it is used by immunologists denotes the same species: isoantibody means an antibody against an antigen appearing in other individuals of the same species—but not in the antibody-producing animal; isoantigens are antigens appearing in some but not all individuals of a given species. Investigators working on trans-
plantation have been using this prefix to denote inbred strains: isologous graft means graft originating from another individual of the same inbred strain. We will use iso in its first, immunological sense. However, we will include under this heading experiments performed within inbred animal strains, because we feel that they belong here rather than under the next heading dealing with autoimmune response.

Isoimmune sera as reagents detecting tumor antigens.—One might expect that isoinmune sera would be better reagents for detecting tumor-specific antigens than heteroimmune sera. Upon isoimmunization, no antibody response is given to species-specific antigens. In addition, it is known from other experimental fields that the effect of isoimmunization with some organs like thyroid (60) and adrenal (57) does not differ from the effect of autoimmune in the production of organ-specific antibodies.

Little work has been performed with human material. Aizawa and Southam (1) showed that antisera of human volunteers with cancer homografts contained antibodies reacting in tanned cell hemagglutination test with extracts of cancer cell tissue culture but not with extracts of tissue culture of normal human amnion cells.

Most extensive animal experiments have been produced by Kidd and his collaborators (14, 29, 32). The authors have shown that rabbits with implanted Brown-Pearce carcinoma form antibodies reacting in complement fixation test with extracts of this tumor but not with extracts of other malignant or normal rabbit tissues. The active antigenic fraction, in spite of revealing many properties similar to viruses, was not infectious. It was believed to be an autocatalytic cellular component with proliferative activity. In his review Hauschka (23) discussed the possibility that some isoantigenic components of the Brown-Pearce sarcoma might have influenced experimental results of Kidd and his collaborators.

Isoimmune sera in tumor therapy.—Gorer and Amos (17) and Amos and Day (2) observed immunity to leukoses in mice given injections of immune sera originating from another strain of mice. On the basis of their findings the authors do not believe that the protective antibody was directed against any histocompatibility antigen. In addition, there are some clinical observations on the beneficial action of blood transfusions from patients with a favorable course of malignancy (52). These data do not seem sufficient to warrant any broader project of immunizing human volunteers with cancer preparations and using their sera for cancer therapy as proposed by Domagk (9).

Resistance to tumors of the same species.—Southam and his collaborators (30) transplanted tissue culture cells to normal human volunteers and cancer patients. No growth of embryonal fibroblasts was observed. Epithelial cell lines which developed a neoplastic character in tissue culture induced cancer nodules in recipients. In some cancer patients recurrence of nodules after biopsy was observed, which was never the case in normal hosts. These interesting results may point to weaker specific anticancer responses in patients, but it still remains possible—and the authors admit this themselves—that the general debility of cancer patients could be blamed for the weaker homograft rejection.

Work on transplantable animal tumors introduced a great deal of confusion until it was realized that the acceptance of a tumor is essentially governed by the same rules as the acceptance of a normal tissue graft (see reviews of Gorer [16], Hauschka [23], and Snell [48]). This conclusion was drawn only after inbred animal strains had been employed in experimental oncology. Extensive experimental work showed that—at least in a vast majority of cases—the immune response to transplanted tumor is not elicited by any tumor-specific antigen but by isoantigens foreign to the host. The tracing of the histocompatibility antigens of II-2 locus (49) was particularly important in elucidating the immune relationship between host and tumor.

However, tumor homograft may be accepted even when it contains some antigenic components which are not shared by the host. The acceptance of a tumor may be visualized as a resultant of two vectors: tumor virulence and the immune response of the host. A tumor will not be rejected wherever the latter is overridden by the first. Upon continued transfer the antigenic composition of a tumor undergoes simplification. Immune mechanisms of the host are probably capable of creating a selective medium responsible for the disappearance of some incompatible antigens from the tumor tissue. To induce the antigenic simplification the immune response of the host must not be too strong; otherwise, the transplanted tumor may be destroyed before the selection of antigenically simplified mutants of the tumor cell population could be achieved.

In experiments of Koprowski (31) ICR mice were made tolerant to C3H tissues; then they accepted C3H ascites tumor to which normal ICR were resistant. After a few passages in tolerant mice the tumor was accepted by normal ICR mice as well. It seems probable that the tolerant mice produced an immune response too weak to destroy
the tumor but strong enough to induce selection of simplified mutant cells.

Certain tumors propagated for many years, like mouse tumors of Ehrlich, Crocker, and Bashford, rabbit carcinomas of Brown-Pearce, and V2 and rat carcinoma of Walker, are now accepted by all animals of the given species (they do not cross species lines). That certainly does not mean that these "non-specific" tumors are devoid of all antigens which may induce iso-immune response in a new host. However, the number of isoantigens is reduced enough not to provoke a response leading to the rejection of a grafted tumor. On the other hand, the tumor will be rejected if the future host is given a chance to initiate the immune response before the transplantation, and this can be achieved by injecting irradiated tumor tissue or even normal tissues.

Whereas in earlier experiments homograft rejection was frequently mistaken for tumor-specific resistance, recent experiments which were performed on inbred mice, under conditions excluding, as far as possible, genetic differences between host and tumor, may point to the existence of a true tumor-specific resistance.

Prehn (38) performed studies on the immunity to dibenz[a,h]anthracene-induced tumors. These tumors, when transplanted to normal isologous mice, were accepted in 81 per cent of the cases. In contrast, transplantation to mice which were previously exposed to the temporary growth of the same tumor was successful in only 43 per cent of the cases. Révész (45) induced sarcoma by methylcholanthrene in inbred mice. Before tumor transplantation mice were twice given injections of irradiated cells of the same tumor originating from the first or second transfer in an isologous host. In the pretreated mice, the tumor was accepted in 15 per cent of the cases as compared with 56 per cent of the control mice. In neither Prehn's nor Révész's experiments did the pretreatment with normal tissue affect the resistance. In addition, the "immune" mice accepted the skin graft from the tumor donor.

It is to be hoped that these interesting experiments will initiate a series of similar investigations, which may offer a final solution to the most important problem of tumor-specific resistance.

AUTOIMMUNE RESPONSE TO TUMOR ANTIGENS

Autoimmune sera as reagents detecting tumor antigens.—The sera of patients as well as of animals with spontaneous tumors could be the simplest reagents for the search of tumor-specific antigens. The main advantage of using these sera is that they do not contain antibodies against normal tissues (not considering natural blood group antibodies). If tumor-specific antigens exist and if they are strong enough to elicit antibody production it should be relatively simple to prove cancer specificity by the reaction between a patient's serum and cancer extract. One would have only to show (a) that normal sera and sera from other-than-cancer diseases do not contain, or very seldom contain, the antibodies in question and (b) that normal tissues and pathological noncancerous tissues do not contain, or only very seldom contain, the antigens in question. To my knowledge these criteria had not been fulfilled either in earlier (10, 25, 26) or in recent papers (13, 20, 36). Normal sera do not provide sufficient control for the reaction of cancer sera. False reactions, not really serological in character (Labilitätsreaktionen of German authors), are much more frequently encountered in the testing of pathological sera than in the testing of normal sera. Similarly, normal tissue should not be used as the only antigen control. Tumor tissue may share some antigens with other pathological tissues without sharing them with normal tissues.

On the other hand, it does not seem probable that the cancer autoantibodies could react with an individual's own tumor without reacting with other similar tumors. All known autoantibodies encountered in human diseases and those experimentally induced in animals are directed against common antigens shared by many if not all individuals of the given species, and an autoantibody has never been described which would react with the individual's own antigens only.

Resistance to autochthonous tumor.—If any autoimmune damage of solid tumor exists under natural or experimental conditions, the mechanism responsible for this damage could be expected to be similar to the mechanisms responsible for organ damage in autoimmune diseases. On the basis of data now available it seems that autoimmune organ destruction experimentally induced in animals is due to cell-mediated rather than humoral immune mechanisms. Humoral antibody may be an excellent indication of autoimmunization, as is the case in experimental thyroiditis; however, its disease-inducing capability is not clear. In this connection it seems plausible that the plasma and lymphoid cell proliferations encountered in some cases of human (4) and animal (3, 37) tumors consist of immunologically activated cells which may be responsible for the growth arrest or even temporary tumor regression. It seems conceivable that some humoral autoantibodies may be responsible for
the defense reactions in leukoses if such reactions exist at all. The destruction of circulating cells may possibly be accomplished by humoral auto-antibodies, which seems to be the case in acquired hemolytic anemia.

There are many unquestionable clinical reports pointing to the dormancy or spontaneous regression of malignant tumors (12, 51). The number of favorable cases is extremely small as compared with the number of cases in which cancer progresses unhindered to a quick death of the patient. However, these observations show that there may exist some defense mechanisms against malignancy and that the organism is not completely weaponless in these diseases.

From the very beginning of immunological research, investigators have been attracted by the idea of increasing the patient's resistance to his tumor by injections of tumor material. This idea was revived in recent years in connection with successful experiments on autoimmune encephalitis and thyroiditis. It was reasoned: If proper methods of immunization may result in the destruction of normal organs, why would it not be possible to achieve the destruction of a tumor in a similar manner?

Witebsky et al. (61) gave injections to twenty patients, in terminal cancer stages, of extracts of the patient's own tumor incorporated into Freund adjuvants. Neither improvement in the clinical course of the disease nor the finding of autoimmune reactions at the tumor site followed by softening of the tumor was observed. Finney and his collaborators (13) immunized nine patients with autologous tumor suspension incorporated into Freund adjuvants. They observed a rise of the antibody titer in the agglutination of red cells coated by tumor extracts as well as inflammatory reactions at the tumor site followed by softening of the tumor and decrease in its size. Fractions I, II, and III of a patient's serum, when injected into metastatic nodules, induced their partial regression. One may hope that these interesting and promising experiments will be fruitful. However, final evidence for the immunological character of the phenomena observed by the Grahams and Finney and his collaborators is still to be furnished.

Of the animal experiments, those recently performed by Klein and his collaborators (30) deserve the most attention. They induced a sarcoma in a mouse by methylcholanthrene; the tumor-bearing leg was amputated, and irradiated tumor cells were injected into the same animal. Two additional injections were made with irradiated material obtained from the same tumor maintained on an isologous host. Then, when the viable autotumorous tumor was transplanted, some degree of immunity was observed. However, some degree of immunity was also observed in mice given injections of normal irradiated tissues, which could hardly be explained in terms of tumor-specific immunity.

The prospect of active immunization as a method of cancer therapy seems still promising. The Freund adjuvant type of immunization, which proved to be efficient in inducing autoimmune diseases, should be employed rather than other types of immunization. Certainly the final outcome of such a procedure would depend on many factors. Most important seems to be the question whether tumors really possess antigens absent in normal tissues. Another question is whether the anticancer immunity, even when already induced, will be able to gain in the time race with the malignant growth.

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