The Transplanted Tumor as a Research Tool in Cancer Immunology*

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Much has been written about the promises, disappointments, and snares for the inexperienced and uninformed in the use of the transplanted tumor as a model in the search for a prophylaxis and immunotherapy of cancer (10, 12, 14, 21, 33, 46, 49, 56). Despite the repeated warnings of the necessary precautions in experimental design, misguided reports of the detection or achievement of "cancer immunity" still appear in reputable technical journals. For the purposes of this conference, therefore, it is necessary to re-examine and re-emphasize the validity, limitations, and pitfalls attending the design and interpretation of transplantation experiments.

The transplanted tumor can be used in two ways in immunologic experiments: (a) as a graft—whose rejection or acceptance is a measure of "tumor-host relationships," and (b) as a ready source of ample quantities of tissue for antigenic assay—the biochemical identity of a given tumor line presumably remaining inviolate from sample to sample. The questions for which we are seeking answers, in terms of the host bearing an autochthonous tumor, are: is there a unique substance (or substances) in the cancer cell which is immunogenic; how is the cancer cell affected by an immune attack; what are the defensive capabilities of the cancer-bearing organism; how are "tumor-host relationships" expressed immunologically?

It is now generally accepted that the conditions governing the acceptance or rejection of a tumor graft are those that determine the fate of normal tissue grafts (14, 38, 45, 46). The genetics and immunology of the "host-graft" relationship are identical for both classes of grafts. Occasional "non-specific" tumor grafts may appear to violate this generalization; nevertheless, it can be shown, by proper experimental manipulation, that the immune response is still in force, though its expression—in rejection of the graft—has been suppressed (46). This will be considered in more detail below.

The basic dicta of host-graft relationships have been most extensively defined by the use of inbred mice and their indigenous transplanted tumors, particularly through the analysis of the "histocompatibility antigens" of the H-2 system (2, 4, 15, 25, 46, 47). (The compatibility, or incompatibility, between graft and host is, of course, a surgically imposed qualification. The organism ordinarily is not called upon to make such distinctions, except perhaps for the "special case" of the "engrafted" fetus.) These antigens appear to be resident in all tissues of the mouse (with possible exceptions); they, therefore, characterize genetically determined differences among individuals (what Jerne [29] has called the "idiom"), not differences between organs or tissues. The demonstration of the universality of the "homograft reaction" and the elucidation of the underlying genetic and antigenic determinants serve to define in precise terms the requirements for establishing the presence of tissue-specific (and by the same token, tumor-specific) antigens (15, 37, 45). Ignorance of these requirements still gives rise to unfounded reports of cancer-specific antigens, cancer vaccines, or immunization against cancer. No doubt the experimental animal, as reported, had been "cured" of its cancer graft (which probably had a precarious foothold from the start), but it would have been "cured," in the same sense, of a graft of normal skin, or kidney, etc.

The transplanted tumor often will pick up viral, bacterial, or fungal passengers, either during the manipulations of grafting, or from resident infections in the host (39, 46). This possibility has been highlighted by current reports on the occurrence of polyoma virus in transplanted tumors (42). The nature of the error that such contaminants can introduce into an antigenic assay is obvious; they may also influence the growth pattern of the car-

* This paper is affectionately dedicated to Professor L. C. Dunn on the occasion of his retirement as Professor of Zoology at Columbia University.
rier graft, either by lowering the graft’s vitality, or depressing the host’s resistance by a superimposed infection. The risk of contamination increases with continued passage of a tumor, and one should be particularly wary with long-transplanted tumors. Various methods have been devised to clear the tumors (46).

There are other variables which can confound an experiment (10, 21, 46). The ambient temperature can affect the rate of growth of a graft, accelerating it with some tumors, inhibiting it with others (28). Operational stress may facilitate “takes” (7); other impinging variables are the age of the host (53) and the site in which the graft is inoculated (17, 19).

The long-transplanted tumor presents another trap for the innocent, of a more fundamental character. I am speaking both of the so-called “specific” tumor, and the “non-specific” tumor, whose lineage may go back to the earliest days of experimental cancer (as examples: the Krebs, Ehrlich, Crocker, and Bashford tumors, and Sarcoma 180, in the mouse; the Walker carcinoma of the rat; the Brown-Pearce and V2 carcinomas in the rabbit). These tumors are “non-specific” (but they do not cross species lines) in the sense that they apparently grow with equal facility in animals of diverse genetic origin—this in contrast to the “specific” tumor which, in the most ideal example, grows only in animals of the same inbred line in which the tumor originated. However, the non-specific tumors are characterized by antigenic specificities, for they will be rejected if the potential host is immunized with the same tumor tissue as the subsequent graft (46). Immunization has been effected by various devices, one being to inject heavily irradiated cells which have thus been rendered incapable of reproduction but remain immunogenic (27, 41). A recent most interesting report (27) describes the induction of immunity to the Novikoff hepatoma in the rat, following the injection of tissue-cultured hepatoma cells which had lost their ability to survive in vivo (were no longer “malignant”) after their residence in vitro.

The source of deception in the use of these venerable tumors is that the initial antigenic identities, which we can be sure characterized both the tumor and the animal which generated it, can no longer be traced, particularly since the tumor’s progenitor was a noninbred animal. Consequently, it is impossible to check antigenic assays of the tumor with assays of comparable normal tissues, the upshot being that the most we are permitted to say of such tumors is that their idiosyncrasies are a reflection of their historical isolation. This conclusion is underlined by the experiences with tumor lines of inbred mice, for it is a common finding that the incidence of “takes” in the first few transplant generations of a new tumor may be a good deal less than 100 per cent, and the growth rate is very slow. However, the incidence will become totality, and there is a marked speed-up of “killing time,” with continued passage. “Antigenic simplification,” and an increase in “virulence” (whatever the underlying mechanisms may be), have been ascribed to such tumors during the course of transplantation (15, 19, 21, 22, 26, 32, 43, 46, 52). In time, these tumors tend to grow in more and more unrelated strains of mice, and, eventually, the distinction between specific and nonspecific becomes blurred.

A further source of differentiation between tumor and indigenous host strain is the genetic drift that undoubtedly occurs in all inbred lines of animals (3, 22, 26, 31, 41, 47) and very likely in the tumor lines also (31, 43, 46). Eventually, the departure between tumor and host is wide enough to cause rejection of the graft. There is, thus, a false sense of security in using normal tissues from the one-time native strain of animals as control materials in the assay for antigenic peculiarities in the long-transplanted tumors. The conclusion, perforce, is that the valid material in the search for “cancer antigens” is the newly arisen tumor (34, 39, 41).

Despite the above account of snares and delusions, the transplanted tumor, when properly used, has elucidated several aspects of cancer immunology; as a concomitant of its very important role in clarifying the more general question of the processes underlying tissue graft rejection. As a prelude to developing this theme further, I re-emphasize that the antigens which we are seeking would have significance to the animal bearing an autochthonous tumor only if they are novel (foreign) to the host, and are immunogenic. The novelty might reside in a “cancer-specific” antigen (or antigens)—i.e., a substance which is private to a given cancer type, no matter from which individual the cancer may come (35, 57); or the substance may be “host-specific,” i.e., private to the one autochthonous tumor (34, 39, 41). The demonstration of either class of antigens could presage an immunotherapy of cancer; the establishment of cancer-specific antigens might permit the development of a prophylaxis.

The utility of the transplanted tumor is predicated upon its biological identity with autochthonous tumors of the same type. There is no evidence to the contrary. “Dependence,” “autonomy,” “progression,” “virulence,” describe attributes of both the original and the derivative
demonstrated both production of circulating isoantibody and sensitization immunogens in the graft--expressed by the solid tumors, fall into two categories--those showing humoral (so-called "cytotoxic") antibody; the sarcomas, carcinomas, and perhaps other classes of phocytes, and most probably other cell types). Leukemias, as a group, are highly vulnerable to ing a fair degree of sensitivity to cytotoxic anti-
combinations. The circumstances underlying this are not known and obviously merit investigation.

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consider the potential vulnerability of tumors to consideration in detail elsewhere in this symposium.

We turn now to a consideration of some of the factors which may be determinants in the quest for an auto-immune response to cancer. In question are the capabilities of the host, and the antigenic stimulus of the tumor, both being aspects of the "host-tumor relationship." The ability to develop an immune response to a tumor homograft appears to be impaired in the host bearing an autochthonous tumor (6, 50, 51,) but there are contradictory reports on how this affects the level of response to heteroantigens (51, 52). Such evidence has only circumstantial bearing on the autochthonous tumor and, of course, would have relevance only if private cancer antigens do indeed exist. A corollary question raised by these observations, but whose consideration is outside the scope of this paper, is the possibility of bolstering the host's ability to respond immunologically. This is being considered elsewhere in this conference.

As for the antigenic stimulus provided by the autochthonous tumor, it may be of such a character (whether due to its quantitative or qualitative aspects) that it engenders an indolent response in the host (such as is sometimes seen with grafts of tumors and normal tissues [1, 9, 14, 24, 45, 46]). The tumor can thereby gain a foothold and eventually "over-ride" the belated defensive reactions of the host. The various inbred strains of (transplanted) tumor (11–13, 19). It is in a com-
parison of properties such as these that the transplanted tumor has made essential contributions to oncology, and the same should apply to cancer immunology.

The development of an immunotherapy must consider the potential vulnerability of tumors to immunologic exposure. Transplanted tumors fall into four general groups, in terms of the consequences of their contact with immunologic reagents (5, 8, 10, 14–18, 30, 32, 36, 44, 45, 48, 55). This classification is based upon the "homograft reaction" and the apparently paradoxic phenomenon of "immunologic enhancement." It is now generally known that the "homograft reaction" is an immunologically specific response--to "foreign" immunogens in the graft--expressed by the production of circulating isoantibody and sensitized cells of the reticuloendothelial system (lymphocytes, and most probably other cell types). Leukemias, as a group, are highly vulnerable to humoral (so-called "cytotoxic") antibody; the sarcomas, carcinomas, and perhaps other classes of solid tumors, fall into two categories--those showing a fair degree of sensitivity to cytotoxic antibody, and those apparently resistant to isoantibody. These relative degrees of difference can be demonstrated both in vitro and in vivo (10, 14, 15–17, 32, 55). The line of demarcation between the latter two groups, however, is not rigid, for it is possible to find an isoantiserum that is highly cytotoxic to a tumor which had appeared to be completely invulnerable to other antisera (55). The difference may be due to qualitative (not quantitative) variance between isoantisera, attendant upon the use of different host-graft combinations. The circumstances underlying this are not known and obviously merit investigation.

Tumors of the latter two categories, but particularly the third, may exhibit enhancement upon contact with humoral antibody, in vitro or in vivo (17, 18, 31, 48). This is expressed as a progressive growth of the homograft, rather than its destruction by the host. As stated, the phenomenon is most easily demonstrated with tumors of the third group, but can be shown with the second by proper manipulation of antiserum dosage, passively transferred (17). It would serve no purpose to speculate here on the modus operandi of enhancement; it is by no means clearly understood and is undoubtedly much more complex than we suspect (17, 31, 48) (Gorer, unpublished data). (Enhancement is yet to be demonstrated as operating with autochthonous tumors, but, if it should, it would obviously be self-defeating to the "auto-immune" response of the host. We are, of course, begging the question here, since we still have to demonstrate auto-antigens in autochthonous cancers.)

All tumors appear to be vulnerable to the sensitized lymphocyte, but it seems, even for those tumors that are apparently unharmed by humoral antibody, that there may be a synergistic action between isoantibody and activated lymphocyte (5). This whole realm of tumor sensitivity is a subject undergoing active investigation. It has direct relevance to the question of cancer immunology, for one of the objectives of our search is the demonstration that the autochthonous tumor is a "homograft," and, as such, could be vulnerable to the consequences of having aroused a "homograft reaction" (34, 39, 41).

To this point we have been considering the manifestations of isoimmunization, which would be of primary concern if there were a possibility of autoimmunity in cancer. The experience with heteroimmune sera is that they are uniformly cytotoxic to all categories of tumors (as classified above) (46). This could hold promise of a "passive" immunotherapy of cancer, if private cancer antigens (whether "cancer-specific," or "host-specific") could be identified and made effectual--an aspect of the problem which will be considered in detail elsewhere in this symposium.

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mice, characterized as to tumor type and incidence, together with the possibility of conducting well-controlled studies with transplanted tumors, are an unrivaled source of material for elucidating these questions. The problem is to develop model experiments, and beginnings have been made (34, 39, 41).

Descriptive terms such as "virulence" and "over-ride" have not been clarified operationally, though they do have direct bearing on our central theme—cancer immunology. The lack of concrete information leaves ample room for speculation, and I may be permitted some also. The increase in "virulence" (as expressed by an accelerated growth rate and an increase in the ratio of "takes"), which is an almost universal accompaniment of continued regrafting of a tumor, has sometimes been attributed to a "loss of antigens." The losses that have been demonstrated (15, 23, 26, 31, 43, 47) involve some of the H-2 antigens of the mouse, and the tumors concerned have been investigated because of their increased capacity to survive in "foreign" inbred strains of mice. There are no reports that a such a mechanism would account for the often observed marked increase in the percentage of successful takes during the early transplant generations of a "new" tumor in mice of its native strain, but the absence of published evidence is more probably due to the lack of any attempts to look for antigenic disparities than to negative findings. It is, of course, possible that an increase in apparent virulence is just that (3, 14) and that it is another expression of tumor "progression" (11).

If the unhindered progress of an autochthonous tumor, or the successful seeding of metastases, should prove to be a consequence of "antigenic loss," then the prognosis for immunotherapy would indeed be gloomy. It has been reported (54) that antigenic substances present in the normal precursor tissues have either decreased in quantity or disappeared during the process of cancerization (which may correlate with the diminution in enzyme content sometimes observed [20]). The significance of this for the etiology of cancer, however, is not known. On the other hand, recent reports (34, 39, 41) would indicate that some types of autochthonous tumors (carcinogen-induced) possess "new" antigens, and that these antigens are "host-specific," in the sense in which the term has been defined above.

What published reports there are of "cancer-specific" antigens are mostly unreliable because they are based on improperly controlled experiments. A possible exception is the "X antigen," demonstrated by Gorer (15, 16) to be present in several transplanted leukemias (but not in all that were examined) of the C57BL/6 strain of mice. Immunity has been demonstrated in inbred mice to grafts of sarcomas newly induced by chemical carcinogens (34, 39, 41). In one report (34), immunity was produced in the mouse in which the test tumor had arisen (though the tumor had been passed through several transplant generations before being implanted back in the original host). There were no marked cross-reactions between tumors arising in several different mice of the same inbred strain—indicating that the antigenic differences between tumor and host resided in "host-specific" immunogens (34, 39).

Finally, I may be permitted a brief excursus into several areas of cancer immunology which are not, strictly speaking, concerned with the transplanted tumor and which will be treated in detail elsewhere in this symposium. My justification is that the problems posed relate to matters which have been illuminated by tissue grafting and that transplantation may play a future role in their clarification. The demonstration of virus-induced tumors in a number of vertebrate species raises the question of whether such viruses might be immunogenic to the host (58). Assuming that they may be, does viral transmission across the placenta, or through the mother's milk, render the recipient "immunologically tolerant" to the agent (in the sense in which "tolerance" is defined for homografts of normal tissues [37]), and thereby incapable of neutralizing it?

In view of the reports of "antigenic simplification," "antigenic alteration," and "antigenic loss," which transplanted tumors exhibit during successive generations of transplantation, might we expect similar happenings in the autochthonous tumor during its "progression," or change from "dependence" to "autonomy," or in the sudden virulent development of metastases? Would an immunologic response developed to the early tumor thus be side-stepped as the tumor developed? The latter question may be asked with respect to the so-called "masking" of viruses. If the tumor virus is the proper target of our immunologic assault, does the vanishing of the virus from the scene make the cancer cell impregnable to attack? This question bears on the mode of action of cancer viruses—about which there is only speculation—for they would have to become an indispensable element to the economy of the cancer cell to cause it damage by immunologic attack directed against the virus.
REFERENCES


The Transplanted Tumor as a Research Tool in Cancer Immunology

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