GUEST EDITORIAL

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Immunotherapy of Cancer in Animals: Models or Muddles? [4]

Immunotherapy, both experimental and clinical, is now fashionable in cancer research. Fashions frequently achieve popularity for irrelevant or inadequate reasons, and they often fade from favor for inappropriate reasons. The former is probably true for immunotherapy. We hope that the latter fate can be forestalled. To that end, we will attempt to answer the following questions: What is immunotherapy? Are animal systems valid models for clinical immunotherapy? What are the imperfections of animal models? How do animal models relate to clinical immunotherapy trials? At the present time, what are the appropriate directions for animal studies?

What is Immunotherapy? [6]

We define immunotherapy as any manipulation that augments the ability of a tumor-rejection immune response to inhibit the growth or spread of an existing tumor. Thus treatments initiated prior to the existence of a tumor are not included, though they may influence the process. The augmentation of tumor-rejection immunity is most directly documented by the development of a greater ability to reject a tumor after therapy than was present before treatment. Local processes may be involved, but the immunity is ultimately systemic and is specifically directed against antigens detectable on tumor cells but not on normal cells. Care must be taken to avoid the "illogism:"

Agent A stimulates immune responses to antigens X, Y, and Z;
Agent A inhibits tumor growth;
ergo, Agent A is an immunotherapeutic agent.

What Does Immunotherapy Have to Offer?

The goal of any cancer therapy is simple—to eliminate all cancer cells in the patient, with as little adverse effect as possible. To accomplish this, the hypothetical ideal treatment would have several characteristics: *strength*—the ability to destroy a relatively large mass of tumor tissue; *specificity*—the ability to destroy malignant cells with little or no damage to normal cells; *systemicity*—the ability to perform discriminative and destructive functions wherever malignant cells may be in the body, regardless of the therapist's ignorance of their presence or distribution. Since we lack an ideal modality, it would be desirable to combine several treatments to achieve complementation of their therapeutic benefits. Unfortunately, the current triad of "conventional" modalities (surgery, radiotherapy, and chemotherapy) all have the same skew to their spectrum of characteristics. They possess strength, but lack adequate specificity or systemicity. The only established treatment with those latter attributes is hormone therapy, which is effective against only a limited number of cancers and is exceedingly weak, because it has no direct cytotoxic effects but operates solely through alteration of cell-cycle kinetics.

Immunotherapy (which cannot now be considered conventional therapy, despite its current vogue) offers the theoretical potential of a systemic treatment with a high degree of specificity and, therefore, low toxicity. In addition, immunologic memory may contribute to post-treatment immunosurveillance against recurrence. These features should constitute the justification for considering immunotherapy as a new weapon in the oncologist's armamentarium. Popularity on other grounds is peripheral to the central issue. "Immunotherapy" which lacks *either* specificity or systemicity has no advantage over simpler, conventional treatments. Nonspecific mechanisms would risk significant damage of normal tissue; this is a major limitation of current therapies. Immunotherapy which is effective only at the site of administration would necessitate treatment at every tumor focus. Currently, the major mode of failure of cancer treatment is our inability to localize minimal, residual tumor, whether it is near the primary lesion or disseminated to the viscera.

Limitations of Immunotherapy

A major limitation of tumor inhibition via tumor-rejection immunity is the relative weakness of the response. At present, it appears that immunotherapy should be restricted to those situations which involve a minimal total tumor mass, such as the residuum that survives optimum conventional treatment. The combination of immunotherapy with other modalities does not necessarily

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[6] We will not list the various types of immunotherapy protocols because they have been reviewed elsewhere (*1–4*), nor will we evaluate any specific animal models.

Editor's note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.

require that the immunotherapeutic maneuvers should be withheld until the failure of the primary treatment is distressingly evident, or even until completion of the conventional treatment. Initiation of immunotherapy prior to surgery, radiotherapy, or chemotherapy, with continuation after the cytoreductive treatment, may facilitate effective immunologic maintenance of remission.

Weakness of tumor-rejection immunity appears to be universal and insurmountable. Even in experimental situations, an immunity which is effective against a small amount of tumor tissue can be overwhelmed by the presence of a larger tumor burden (*5, 6*). This does not mean that the immune system is intrinsically limited in its ability to destroy malignant tissue. Large masses of tumor tissue can be effectively rejected if they possess the proper antigens (*7*). So the weakness of immunotherapy is due to the nature of cancer-related antigens, not to an incompetence of the immune machinery per se. The elusiveness of tumor-rejection antigens is further compounded by the fact that neoplastic progression may permit tumors to become even less antigenic than they were at the outset (*8, 9*). Thus immunotherapy shares with drug and hormone therapy the problem that resistant variants may arise and become predominant through selective inhibition of sensitive cell populations.

Are Animal Systems Valid Models for Clinical Immunotherapy?

To answer that question, we need to consider what a model is. Webster defines a model as "a description or analogy used to help visualize something that cannot be directly observed," or "a miniature representation of something" (*10*). Thus a model is a substitute for something but is not equivalent to its object. A model differs from its object in some respect(s). A commonsense example illustrates that there can be many kinds of models of the same object. An aircraft may be modeled by a construction mock-up, which reproduces most morphlogic details, including size, but is largely nonfunctional; by a radio-controlled recreational model, which differs in size and detailed morphology, but which can fly; by a wind tunnel model, a proportionately scaled-down replica of the exterior, able to faithfully reproduce all aerodynamic features of the real plane, but wholly incapable of flight; or by a variety of other "representations," each with a unique set of differences from the object. Although each such model presents valid information about the object, each is unable to represent certain other aspects of the aircraft. Thus any model is intrinisically an imperfect or incomplete representation of its object.

In that sense, animal tumor systems can be used as models for immunotherapy in humans. Although they are imperfect representations, the discrepancies between animal and human immunotherapy may be minimized by careful selection of a tumor–host system and by proper experimental design. Some discrepancies will be irreducible, but they should be identified as carefully as possible and must always influence interpretation and extrapolation of results.

What are Imperfections of Animal Models?

Table 1 lists some of the discrepancies between animal models of immunotherapy and the human cancers they purport to represent. None of these differences is universal among animal models, yet no model is free from such problems. Basically, animal models incorporate features of convenience for the experimentalist, a luxury not available to the clinician. Animal tumors are commonly implanted into sites not representative of the natural history of the cancer (e.g., beneath the skin, although skin lesions are not a major problem in humans). Successful models may depend on syngeneic animals as a source of tumor cells for antigen or as a source of sensitized cells, whereas for humans the supply of such materials is limited to the patient's own tissues. Even the most artificial model has the potential to produce new information about the immunobiology of cancer. However, if preclinical investigations are to generate guidelines for the efficient design of appropriate clinical studies, every effort should be directed toward reduction of the discrepancies.

TABLE 1.—*Common discrepancies between animal models of immunotherapy and human neoplasia*

Common characteristics of animal models	Relevant features of human neoplasia
Biologic features of tumors	
Transplanted	Primary, autochthonous
Short latent period	Long latent period
Rapid growth	Slow growth
High growth fraction	Low growth fraction
Sarcomas and leukemias predominate	Carcinomas predominate
Advanced stage of neoplastic progression	Relatively early in neoplastic progression
High antigenicity	Low antigenicity
Infrequent metastases, usually regional	Metastases common, frequently visceral
May carry passenger viruses	Viruses uncommon
Host characteristics	
Young adult	Aged or early childhood
Healthy	Frequently debilitated
Inbred	Outbred
Small body size	Large body size
Short life-span	Long life-span

Perhaps the greatest problem in the field of experimental immunotherapy is the paucity of evidence to establish the general applicability of "principles" developed in animal studies. *Effective* immunotherapy models (tumor–host systems, procedures, and agents) tend to be studied exclusively in a single laboratory, which reminds one of the state of viral oncology 30 years ago. The value of such unilateral investigations can only really be appreciated as observations are confirmed in other laboratories. To this end, there is a serious need for both investigators and editors to maintain a balanced presentation of results, *including negative studies and failure to confirm results of other studies.*

A second basis on which to support the general applicability of experimental models is to avoid reliance on a single tumor line or primary tumor system. Comparative studies in several tumor models will identify results that are peculiar to a single system and will confirm observations with general or clinical applicability. A panel of models should represent a spectrum of tumors in terms of histology and etiology, as well as host species. This will minimize conclusions based on model idiosyncrasies. Another criterion of model selection involves the relative merits of primary tumor as compared with long-transplanted tumor lines. The transplanted lines have the advantage of stability, reproducibility, and a fund of information concerning biologic behavior. In contrast, studies which involve primary tumors, especially in outbred hosts, more closely approximate the variability and unpredictability characteristic of human neoplasia, as well as the features of neoplastic progression, immune surveillance, and spread which may be unique to in situ

primary cancer. The most comparable situation would be the study of naturally occurring tumors in the hosts of origin, but that is usually impractical unless it is based on extensive preliminary work with transplanted tumors. At the very least, immunotherapy must be studied in syngeneic systems.

The experience in other areas of oncology has identified two pitfalls to be avoided in developing animal models of immunotherapy. For many years, experimental models used for cancer chemotherapy screening were selected primarily for their convenience in the laboratory (rapid growth and high growth fraction). Drugs selected in such systems have been highly effective against the few human tumors that shared those features but are less effective against the more common, slowly growing human cancers. Similarly, bioassays for carcinogenicity have been efficient in confirming the oncogenic potential of agents under suspicion due to human epidemiologic evidence but have been less valuable in identifying unanticipated hazards to humans.

How do Studies in Animal Models Relate to Clinical Immunotherapy Trials?

Animal tumor models are substitutes for the real thing—human cancer. Therapeutic trials in human cancer patients and studies in animal tumor models are not mutually exclusive, nor should they be antagonistic. Wherever and whenever important questions about management of human cancer can be answered by clinical experimentation or trials, that should be done. Any treatment that consistently and significantly prolongs survival of human cancer patients should be studied in patients and not in animals. Unfortunately, trials of immunotherapy of human cancer have not produced such clear-cut benefits. A large number of agents and protocols have been tested, but effects have been weak, rare, local, or unpredictable. Usually, the approach has been empirically selected from a variety of possibilities. Anecdotal reports have tended to emphasize the rare, dramatic "responses;" it is not clear whether such results have been therapeutic or spontaneous. Trials with retrospective controls suggest that current treatment, including immunotherapy, is better than earlier methods, but they do not tell whether immunotherapy is the only, or the relevant, improvement. Such studies certainly permit the conclusion that immunotherapy, as tried to date, will not be a panacea. Randomized, controlled clinical trials of immunotherapy have been rare. Although they represent the most important, definitive means of establishing improved modes of therapy, they should be the capstone of the evaluation procedures, not the inital screen.

The numbers and types of questions that can be answered by controlled clinical trials are limited. Those limitations are the very area in which animal experimentation offers significant advantages. Any clinical trial, controlled or otherwise, *should* be limited by ethical considerations, ultimately in the form of an investigational new drug or clinical investigation committee approvals. New treatments must be tested initially in patients with advanced disease, and then only in combination with "best available" conventional therapy. However, animal studies permit new approaches to be tested individually as primary treatments and to be compared with the results of withholding all therapy. In animals, it is possible to study large numbers of subjects, and thereby attain greater sensitivity in the detection of therapeutic benefits, better control of experimental variables, and greater capacity to evaluate numerous variables. Assessment time in animal models is short, thus there is less "turn-around" time for confirmation and refinement of the study. Overall, these features establish animal models as an appropriate first level for evaluating new therapies. They can provide direction in the selection of agents and procedures for clinical testing and can help to refine the questions that must ultimately be answered by human experimentation. Thus studies in animal tumor models and therapeutic trials in human cancer patients are complementary and interdependent. The limitations of clinical experimentation and problems of patient care define the goals and relevance of preclinical, animal model studies; the latter, in turn, can focus the efforts expended in human research.

Given the interrelationship of animal and human experimentation, one is confronted with the problem of extrapolating results in animals to the clinical setting. We feel that there are four approaches to the solution of that problem: 1) General relevance of animal results should be established by application in several animal models, representing different tumors and species. Idiosyncratic behavior of systems and agents should be revealed by this strategy. 2) General relevance is further supported by confirmation of results in different laboratories. 3) An understanding of the basic mechanisms by which immune stimulants inhibit tumors (and why they fail in some situations) may assist in the prediction of the clinical value of certain agents or in the design of new ones. 4) Finally, principles of immunotherapy derived from animal models should not be adopted as the basis for routine clinical therapy without confirmation in controlled clinical trials.

What are Appropriate Directions for Animal Studies of Immunotherapy?

Many of the immediate needs in the area of experimental immunotherapy have already been identified in the preceding discussion. Successful animal models need to be confirmed in other laboratories and unsuccessful work should be communicated. Working models should be modified to more faithfully represent the features of human neoplasia. Beneficial treatments should be evaluated in several animal tumor systems. Those treatments which have general applicability should be studied for their mechanisms of action. This would identify those aspects of the immune response which correlate with curative procedures. Furthermore, such analysis should include comparisons of effective and ineffective procedures and responsive and unresponsive models. Such data would develop some basis for *predicting* the efficacy of a given procedure in a given clinical situation. Studies of mechanisms of action should be supported by in vitro analysis of immune responses to identify those tests which may be useful predictors or monitors of the in vivo benefit of immunotherapy. There is a need to identify agents and procedures with potential for synergistic action and to define which types of immunotherapy will work in combination with the more conventional modalities. One approach would be to identify chemical and biological characteristics of immune stimulants and of tumor antigen preparations that contribute to tumor inhibition in vivo. Little is known about the permanence of immunotherapeutic tumor regressions (*11*) or how to avoid late recurrence in individuals "cured" by immunologic means. Finally, with the development of meaningful animal models, immunotherapeutic procedures

(immune stimulants, tumor antigen preparations, and administration protocols) can be compared for their relative effectiveness.

A new generation of animal models of cancer immunotherapy is needed, as is confirmation of much work in existing models. This challenge has been well put in the following exchange (*12*).

"When BCG trials were started in man . . . the rationale seemed to be that it was permissible to attempt to benefit some patients and to ignore the likelihood that others would be harmed . . . The necessary background information should have been a pre-condition for the initiation of trials with living BCG in man." (D. W. Weiss)

"I would press onto laboratory scientists the fact that when human disease is bad, as in acute myeloid leukemia and melanoma, the clinician and the clinical investigator feels the pressure of the bad prognosis. He is not willing to stand around and wait for the animal models to be perfected before beginning carefully conceived efforts to use the best ideas in therapeutic trials." (R. A. Good)

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