

Immunotherapy: Bewitched, Bothered, and Bewildered No More

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The field of immunotherapy holds clear promise not only for the development of new approaches to cancer and other diseases, but also for providing fundamental insight into the human immune response. In order for this promise to be realized, however, the scientific community must overcome an array of challenges. These challenges reflect not only the difficulties inherent in conducting investigations in human patients, but also difficulties created by the culture and practice of our own institutions, reward structure, and funding mechanisms. We suggest steps to be taken to reinvigorate basic research in human subjects as part of the mainstream of science.

Introduction

Immunotherapy refers to any approach aimed at mobilizing or manipulating a patient's immune system to treat or cure disease. Although the term has been most often associated with therapies for established malignancies (1–6), immunotherapy is of increasing interest as an approach to arrest cancer at a much earlier stage (7). In addition, as illustrated in the accompanying articles, immunotherapy is pertinent to the investigation and treatment of transplantation, autoimmunity, chronic inflammation, and infectious disease. In general, the strategies range from therapeutic vaccines that mobilize a patient's own immune system *de novo* (so called “active” immunotherapy), to administration of preformed biological reagents such as monoclonal antibodies, cytokines, or previously activated immune cells (“passive” immunotherapy) that deliver or modulate a specific arm of the systemic immune response. We will use the example of cancer to illustrate the potential of these approaches and, while we ourselves are primarily laboratory scientists, to stress the need to make patient studies a more active component of basic research in immunology.

Our view is that scientists interested in human immunotherapy research are often left in the position of the confused romantic in the Rodgers and Hart song “Bewitched, Bothered, and Bewildered” (Fig. 1). Immunotherapy was initially “bewitched” by promising discoveries in mice that failed to translate to humans, then “bothered” by burdens involved in studying immunity in humans, and is currently “bewildered” about how to stimulate and pursue a new human direction in immunology.

The field of immunotherapy is often char-

acterized by the term “translational research.” Although this term telegraphs a well-intentioned desire to directly transfer basic discoveries from the laboratory to the clinic, it also connotes a problem. “Translation” ignores the fact that investigation in the clinic and the laboratory is a two-way street, with information learned in humans often generating new avenues of investigation in model organisms, such as mice. Moreover, and of equal concern, “translation” implies that basic principles learned elsewhere (often in mice) are directly applicable to humans. If only it were this simple.

To be successful, immunotherapy requires a broadening of basic research in humans. Not enough is known about how the human immune system works or how it responds, not just to cancer but to many other diseases, such as autoimmune disorders and allergy. For example, spontaneous human malignancies differ fundamentally from experimental mouse tumors (8), and the human and mouse immune systems differ considerably from one another (9). Equally important, having therapy as the only aim of human immunology research overlooks the need to understand the properties of the human immune system that resist cancer or allow the disease to progress. We

need an approach that fosters the pursuit of basic discovery in the clinic and eliminates the basic versus applied distinction implied by the term “translation.”

Encouragingly, immunotherapy lends itself to such basic research in patients, since the immune system can often be assessed and even manipulated with relatively few risks. In the case of cancer, three known features of the disease set the stage for basic discoveries in immunology. First, the changes that drive cancer, often genetic, also generate new antigens that can be and are recognized by the immune system. Second, tumor cells as well as their supporting vessels and environment (stroma) can be highly sensitive to, and also can alter, the array of immune cells and their products. Third, the progression of cancer may rely upon direct or indirect evasion of the immune system by the



Fig. 1. TIME cover 26 September 1938 of composer Richard Rodgers and lyricist Lorenz Hart. [Credit: Time Inc./Time Life Pictures/Getty Images]

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Table 1. Some of the intrinsic obstacles to human research.

- Outbred nature of the study population
- Difficulty of access to critical tissues
- Intensity of protocol design
- Demands of protocol management, record keeping, and sample collection
- Concerns and needs of patients
- Time required per experiment

tumor cells. Our view is that the time is ripe to obtain a better scientific understanding of these features in patients and ensure a more comprehensive view of cancer and other diseases. To do so, we first need to acknowledge the obstacles and then bring about some changes.

Bewitched: The Early Allure of Immunotherapy

The field was captivated by early provocative experiments in mice. Many of these demonstrated that immune manipulations, such as the injection of newly isolated cytokines, brought about the regression of existing tumors. This work harkened back to even earlier human studies by William Coley in the 1890s, who injected microbial adjuvants and on occasion induced tumor regression (10).

In the modern era, the first human studies “translated” from the mouse work were disappointing. For example, although interleukin-2 (IL-2) treatment was effective at eliminating the B16 melanoma from mice, even high doses of IL-2 (or lymphocytes treated with IL-2 *ex vivo*) were of little or no benefit in most cancer patients (11, 12). Based on such negative results, many tended to dismiss the therapeutic potential of immunotherapy. To us, such a reaction was unwarranted, because it had been based on expectations that what was effective for treating experimental tumors in mice would be immediately relevant to treating spontaneous tumors in humans. Instead, the mouse should be regarded as an excellent starting point for research designed to understand the human response to diseases such as cancer; experimental models should provide conceptual guidance rather than assurance and specific targets.

Of course, the degree of difficulty for understanding the immunology of cancer is considerably higher in humans than in mice. One cannot rely on relatively quick readouts, such as monitoring tumor nodules growing rapidly in inbred mice, since in humans one must confront a complex, long-lasting metastatic disease that develops in genetically dissimilar subjects. Thus, as in all other areas of science, there can be no substitute for studying unknowns in the most direct fashion possible in humans.

Bothered: Overcoming the Obstacles to Human Research

Bewitched or not, human-oriented research is hindered by obstacles both intrinsic and ex-

trinsic. The intrinsic barriers relate to the unique features of human beings as experimental subjects and the regulatory requirements for this human research (Table 1). For most conditions, the clinical investigator is not able to access internal tissues (e.g., lymphoid organs) or to use routine analytical methods (e.g., immunocytochemistry) nearly as easily as in laboratory studies. It is both-ersome to know what you would like to measure but cannot, or at least not without great difficulty. Nonetheless, for immunotherapy, many new assays do exist for careful monitoring of immune status, e.g., MHC (major histocompatibility complex) tetramers, peptide libraries, and cytokine and gene arrays (13, 14). High-resolution, noninvasive *in vivo* imaging methods are also beginning to emerge (15).

Other intrinsic obstacles are less scientific, but no less bothersome. Currently, the individual investigator must deal with endless yet essential nonresearch tasks that consume time and interfere with the ability to do experiments. Here, appropriately committed and focused institutional infrastructures might quickly work wonders. Clinical research requires a team effort, comprising not only clinical practitioners and laboratory scientists, but also other professionals dedicated to solving the many regulatory and logistical problems that must be met. Dedicated professional assistance would allow individual investigators to concentrate on the patients, the disease, and the experiments.

Let us turn to the extrinsic obstacles to human research (Table 2). These need not exist, and many appear to emanate from our own scientific culture. Many elite, basic science journals often consider valuable human studies to be inadequate because they lack the mechanistic depth we have come to expect from studies using laboratory organisms. Often precluded from publishing the best of human research in such journals, clinical sci-

entists struggle for professional advancement after having been excluded from the scientific mainstream. As editors ourselves, we now feel that basic research journals should get equally excited about hard-earned advances in understanding valid scientific problems as they exist in humans, trading some expectation of mechanistic insight for inherent relevance and respect for the demands of working within a proscribed lengthy human protocol (16). With broader communication of important human results, studies on mechanism can begin.

Peer review for funding in human research remains another extrinsic obstacle. Productive patient-oriented researchers are often evaluated not by peers, but by scientists who study simpler systems. In our view, new peer review bodies dedicated to human research are desperately required. Likewise, academic review within individual institutions will benefit from additional perspectives to evaluate human research; appointment and promotion cannot rely entirely on traditional academic standards of pace and place of publication.

Departments of medicine, surgery, pediatrics, and the like are logical homes for an expansion of human researchers who interact with and learn from, rather than mimic, their nonclinical counterparts. By enhancing the image of basic human research, increased numbers of laboratory scientists also will be inspired to join interdisciplinary teams devoted to complex problems. Such cooperative efforts would strengthen the scope and depth of human research to the benefit of clinical and laboratory scientists alike. Contributions to team efforts must be considered at times of promotion, whether those contributions involve clinical work, laboratory work, methods development, or infrastructural support. Enthusiastic leadership is required to ensure that credit is shared widely.

Another extrinsic obstacle pertains to the reagents needed for study. The requirement for good manufacturing practice (GMP)-grade material in human research is absolute. GMP cytokines and antibodies, however, cannot simply be bought off the shelf; nor can cells or cell products simply be produced in one's own laboratory. The limited availability of such reagents, combined with their great cost, is often controlled by companies and/or patent holders who do not make

Table 2. Some of the extrinsic obstacles to human research.

- Intransigence of elite basic journals with human studies
- Inequity of peer review in study sections
- Inappropriate criteria for academic promotion
- Lack of emphasis in clinical departments and health care institutions
- Poor access to GMP reagents required for study
- Shortage of funds for investigator-initiated human research
- Paucity of mentors and young patient-oriented researchers

them accessible to researchers. Although economic considerations may in part explain this situation, we need to find creative ways to overcome such counterproductive and often drastic impediments to the progress of academic research. In the interim, we need infrastructure support to help negotiate reagent availability.

A reorganization in funding to invigorate patient-oriented research has been urged (17, 18). The recently proposed NIH Road Map indicates that \$8.4 billion of NIH funds already are spent annually on clinical research (19). This is an impressive amount, but it is unclear how much of this sum is devoted to large-scale trials of clinical efficacy and outcome, as opposed to investigator-initiated basic research in patients. Will the best ideas for immunotherapy emerge without fundamental research on patients? We think not, and the slow pace of success in achieving new preventions and therapies for cancer, let alone HIV and malaria, despite fantastic progress in the laboratory, would appear to bear out this opinion.

Most importantly, we must foster the investigators (and mentors) who are ready with creative ideas and preliminary data before an invigorated human research enterprise can grow. Many foundations have begun to emphasize support for young investigators committed to human research, such as the Burroughs Wellcome, Damon Runyon, Charles A. Dana, and Howard Hughes Medical Institute. Gratefully, organizations such as the Ludwig Institute for Cancer Research and the NIH are also rising to support human research, especially for groups of investigators whose complementary skills enable the formation of teams (laboratory scientists, clinical scientists, infrastructure support professionals) required to tackle tough problems in immunotherapy.

Bewildered: Challenging Science Waiting to Be Pursued

There are many challenging unknowns in immunotherapy, so that it seems to us bewildering they are not being investigated more intensively in humans. As already mentioned, research in this field is directed by three major features of cancer: the presence of mutations and other changes that provide cues for the immune system to recognize, the sensitivity of tumor cells and their supporting stromal elements to the activities of immune cells and their products, and the capacity of cancer cells to evade, influence, and exploit the immune system at multiple levels.

An example of new principles emerging from the study of patients is multiple myeloma, a tumor that can be studied more directly than most because its cells reside accessibly in the bone marrow. In patients with advanced disease, T cell responses to the cancer

cells cannot be detected, but when properly stimulated, T cells from these patients are not irreparably silenced but can be induced to develop into tumor-reactive killers (20). In contrast to the situation in advanced disease, tumor-reactive T cells can be readily identified in fresh bone marrow samples from patients with premalignancy (21). Coupled with new evidence that premalignant tumor cells show many similarities to those in advanced disease, the human studies make a case for more intensive research on host responses in premalignancy, which in turn suggests the possibility of therapeutic vaccination in cancer (22).

An active new area of immunology in humans is the regulation or suppression of immune function. Following stimulation, lymphocytes initially expand rapidly, after which cell expansion and function are controlled by multiple pathways, many of which are only just being identified and have thus far been characterized primarily in mice. These include the B7 family and PD-1 ligands of activating and inhibiting lymphocyte receptors, as well as several classes of suppressor lymphocytes (23, 24). By pursuing these regulatory pathways in patients, one should be able to understand their physiological roles and chart protocols that might harness their potential therapeutic benefit.

Our own interest is in dendritic cells (DCs) because of their critical roles in both innate and adaptive immunity (25). Two sets of DC functions nicely illustrate the potential of these cells for research that seeks to mobilize the immune system in a tumor-specific manner. First, DCs have distinct and regulated mechanisms to capture antigens, especially rich sources of antigens like tumors, and present these to lymphocytes (26). In other words, the numerous genetic changes in cancer cells that provide targets for tumor-centric therapies might also provide an opportunity for immunological targeting focused through the DC. Tumor cells likely express a panoply of new antigens for DCs to present, potentially eliciting different classes of tumor-resisting lymphocytes. Second, DCs sense the environment; for example, in some settings, DCs enhance the formation of cytotoxic (killer) T lymphocytes, while in others, DCs induce antigen-specific immune silencing or tolerance. These areas can be addressed in cancer through research on DCs, for example, those that are loaded *ex vivo* with an array of tumor antigens and then reinfused into the patient (5), or possibly by manipulating DC function directly *in vivo* (27). A move from the injection of tumor antigens and adjuvants empirically, as in past immunotherapy studies, to more precise targeting and maturation of properly positioned DCs should prove valuable in the future.

To summarize, several questions can now be studied concerning the responses of patients to cancer. Which antigens are recognized (28, 29)? Will cell-based, protein, nucleic acid, or viral vector vaccines be most effective, and in what settings? Should immunotherapy focus on defined antigens shared among tumors, or on whole tumor cells that may carry many patient-specific alterations (20)? With proper immunization, can cancer-causing mutations be targeted by potent cellular resistance mechanisms (30)? Is the immune system ignoring the tumor, and capable of being awakened at the level of dendritic cells (5), or is the tumor capable of active silencing or tolerance (31)? Can one improve access of activated immune cells to tumors, and once there, are the immune effector mechanisms capable of destroying cancer cells faster than the cancer cells are growing (32)? Are regulatory and suppressive pathways exploited by tumors to evade the immune response, and can these pathways be manipulated to increase resistance (33, 34)? If the immune response can be expanded, will subsequent tumor-evasion mechanisms need to be nullified in tandem (35)? Can the innate and adaptive responses be harnessed not only against the tumor, but also against its supporting stroma of connective tissue, vessels, and inflammatory cells (36)? Research on these questions in humans can be expected to reveal surprises and more systemic understanding of disease.

Conclusions

Bringing immunology to medicine offers exciting and concrete scientific challenges. By addressing such challenges in immunotherapy, the multiple responses of the human immune system will be revealed, and research will better provide potent and antigen-specific treatments. Although we have outlined some problems and potential benefits of cancer immunotherapy, the analysis of the human immune system should provide insights and therapies for a range of other disorders, as discussed in the accompanying articles. One need consider only the dramatic benefit that accrues in Crohn's disease and rheumatoid arthritis by treating patients with agents that attenuate levels of tumor necrosis factor- α (37). The same situation applies to new monoclonal antibody therapies for malignancy (4). The clinical targets in immunotherapy are reminders of how much needs to be learned to enhance responses (in infectious diseases and cancer) or to silence immunity (in transplantation, allergy, and autoimmunity) in a disease-specific manner.

Human immunotherapy is a new direction, not a nostalgic return to an earlier era. The stage has been set, however, by decades of knowledge gained from basic science in mice and other experimental organisms. This basic discovery engine must be protected and enriched.

The extent to which immunotherapy becomes a valid discipline will depend on new sources of funding to invest in or capitalize upon the laboratory successes that have already been "purchased" with public funds. For one thing, distinct new structures must be built to overcome the obstacles to human research. The demand for human research cannot help but benefit patients, taxpayers, and pharmaceutical companies alike.

Perhaps even more than new funds, there is a need for change within the scientific community. If we are to attract and cultivate exciting young investigators, we must create a reinvigorated scientific culture that regards basic discoveries in the clinic as valid and esteemed as discoveries at the laboratory bench. If we can accomplish this, just as the song ends, we will be "bewitched, bothered, and bewildered no more" (38).

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38. Bewitched, Bothered, and Bewildered. Lyrics by Lorenz Hart, music by Richard Rodgers; from the musical "Pal Joey" (1940).
39. C. Moberg provided important expertise in preparing the manuscript. R.M.S. has been a consultant for Merix, a company devoted to using RNA-transfected dendritic cells in immunotherapy. I.M. is on the Scientific Advisory Board of Cellular Genomics, Inc., a biotech company involved in small molecule approaches to immunotherapy.

REVIEW

Cancer Immunotherapy: A Treatment for the Masses

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Cancer immunotherapy attempts to harness the exquisite power and specificity of the immune system for the treatment of malignancy. Although cancer cells are less immunogenic than pathogens, the immune system is clearly capable of recognizing and eliminating tumor cells. However, tumors frequently interfere with the development and function of immune responses. Thus, the challenge for immunotherapy is to use advances in cellular and molecular immunology to develop strategies that effectively and safely augment antitumor responses.

Ambrose Bierce's cynical description of the evolving field of medicine as "a stone flung down the Bowery to kill a dog on Broadway" often aptly describes conventional cancer therapies. By contrast, the immune system has evolved strategies, largely in response to infections, to efficiently search for and specifically destroy diseased targets. After nearly a century of debate as to whether the immune system can actually target tumors (1–3), compelling evidence now suggests that immune cells play an important role in the

control of malignancy (4). This has first been implied by both occasional spontaneous regressions of cancers in immunocompetent hosts and increased cancer incidence in immunocompromised individuals. Second, tumor immunity can be demonstrated in experimental animal models. For example, mice with defined immunological defects exhibit greater susceptibility to spontaneous and induced tumors, with many of these tumors rejected if transplanted into normal hosts (4, 5). Third, the immune system often appears cognizant of tumors, as reflected by an accumulation of immune cells at tumor sites, which correlates with improved prognosis (6). Finally, with improved technologies, antitumor immune responses can now be detected directly from many patients. Augmenting these responses has started to yield therapeutic benefits not only in experimental models

but also in cancer patients. Advances in cellular and molecular immunology in the past two decades have provided enormous insights into the nature and consequences of interactions between tumors and immune cells and continue to suggest strategies by which the immune system might be harnessed for therapy of established malignancies.

Cells of the innate immune system respond to "danger" signals, which can be provided by growing tumors as a consequence of the genotoxic stress of cell transformation and disruption of the surrounding microenvironment. Under ideal conditions, these signals will induce inflammation, activate innate effector cells with antitumor activity, and stimulate professional antigen-presenting cells (APCs), particularly dendritic cells (DCs), to engulf tumor-derived antigens and migrate to draining lymph nodes to trigger an adaptive response by T and B lymphocytes. Despite this well-orchestrated surveillance operation, the presence of a tumor indicates that the developing cancer was able to avoid detection or to escape or overwhelm the immune response. Progressing tumors often exhibit strategies that promote evasion from immune recognition (7), such as physical exclusion of immune cells from tumor sites, poor immunogenicity due to reduced expression of ma-

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