Translating mouse models into clinical therapies has never been easy. Immunotherapies for cancer also have to contend with an approval process designed for testing drugs. It may be necessary to consider different endpoints and objectives when evaluating the efficacy of these newer approaches.

Immunotherapy of human cancer: lessons from mice

Pramod K. Srivastava

Center for Immunotherapy of Cancer and Infectious Diseases (MC1601), University of Connecticut School of Medicine, Farmington, CT 06030-1601, USA. (srivastava@nso2.uchc.edu)

Studies on the cancers of mice inaugurated the formal analysis of cancer immunity and broadly outlined the interplay between cancer and the immune system. Translation of that understanding into clinical reality has been and remains difficult and is the subject of this commentary. Experimental exploration of cancer immunity in mice led to the identification of heat shock proteins as chaperones of antigenic peptides and raised the possibility of their use in the immunotherapy of human cancer. I describe here some of the lessons learned from the pursuit of that possibility, discuss the process through which some key questions were addressed in the early clinical trials and detail how the successive rounds of trials were initiated. This process reflects some of the challenges posed by immunotherapy, as opposed to chemotherapy, and other challenges that are unique to immunotherapy with heat shock proteins. Heat shock protein vaccines must be produced for each patient from that patients' cancer. Such autologous protein therapy has no precedent in the history of clinical development. Hence, new ground had to be trodden, new rules devised.

Background

The observation that heat shock proteins isolated from cancers of mice and rats elicited specific immunity to the cancers from which they were derived, led to the discovery that heat shock proteins are associated with peptides, including antigenic peptides[1]. These peptides, in association with the heat shock proteins, elicited potent and specific T cell immunity to the cells that presented them. The mechanism behind this phenomenon turned out to be pretty much as it was originally proposed[2,3]. Across species, heat shock proteins are the most highly conserved group of molecules, as is the receptor for at least one of them[4], and so a key question was whether heat shock protein–peptide complexes could elicit protective immunity to human cancers.

Although I described the essential phenomenon as early as 1982–1986[5,6], clinical exploration of heat shock proteins did not begin until more than a decade later. This was not for lack of clinical opportunity but was a deliberate and personal decision to wait until the mechanisms responsible for the immunogenicity of heat shock proteins became clear (in 1993–1994). The first clinical trials to be initiated and completed included those treating gastrointestinal, pancreatic and renal carcinomas and melanomas. In each of the trials, preliminary evidence of immunological or clinical activity was obtained[7–9]. Formal evidence of clinical efficacy is now being sought through one ongoing and other soon-to-be-initiated Phase III trials.

Surrogate marker—a philosopher's stone?

The first, and arguably the most difficult, question that needs to be addressed for human trials was to do with measurement. How does one measure the anticancer activity of a vaccine? In mice the answer is straightforward: cancer-bearing mice are immunized and the results quantified by measuring tumor size and survival. This process, although cumbersome and time-consuming (typically, a complete experiment takes 4 months), is feasible. When mice with measurable, large tumors are treated with heat shock protein–peptide complexes, there is a deceleration of tumor growth, stabilization of tumor size and, rarely, tumor shrinkage. The most dramatic antitumor activity of heat shock proteins is observed where mice have been rendered apparently disease-free by surgery but are still at a 100% risk of death due to recurrence of metastatic cancers. In such situations, treatment with heat shock protein–peptide complexes protects an overwhelming majority of mice from the recurrence of cancers[10] (**Fig. 1**).

If one were to apply to human trials the same measurement of efficacy as is used in mouse studies, a 4-month experiment with mice would translate into a decade or more of clinical exploration. A first, safety, trial using various doses would easily take a year. A Phase II trial, to actually measure antitumor activity *in vivo* at various doses, would take another 3–4 years, as patients would have to be accrued and then followed up for a sufficiently long period of time after treatment to obtain a clear sense of activity. Finally, a randomized study with several hundred patients could take up to 5 years or more to complete.

For this reason one looks for a surrogate marker, one that can be measured early and is predictive of the clinical outcome. In light of the overwhelming evidence that T cells play a critical role in antitumor immune responses, one would imagine it would be possible to simply measure T cell responses before and after immunization to obtain a fair measure of whether or not immunotherapy was going to succeed. However, despite extensive immunological analyses of tumor-bearing mice and cancer patients, no *in vitro* correlates of tumor-protective activity have been found by us or by others (**Fig. 2**). Mice that are undergoing (successful) immunization do not necessarily show distinctive T cell responses as measured *in vitro*, and mice that show distinctive T cell responses are not necessarily protected from cancer[11,12]. Experience from human cancers also shows little correlation between true anticancer activity *in vivo* and immune responses measured *in vitro*[13–15]. It is clear that we do not yet understand the rules that govern T cell function *in vivo* and therefore cannot reproduce that function *in vitro*.

These ideas were first discussed in 1996[16], but have acquired renewed validity in light of our collective clinical experience since then. Hence, one has little choice but to rely on *in vivo veritas* in clinical development as in studies with mice. Approaches that can gauge immune responses *in vivo* without manipulation *in vitro*—such as the use of major histocompatibility complex (MHC) class I–peptide

tetramers, which measure the number of specific T cells *in vivo*, or SEREX (serological analysis of recombinant cDNA expression) analysis[17], which assesses a patients' antibody responses to a broad range of molecules—are promising developments for monitoring immune response. However, their validity as true surrogate markers remains to be shown.

It will be instructive to look at the question of surrogate markers in chemotherapy studies. The success or failure of chemotherapy has generally been judged by the ability of the drug to engender a "response", defined as partial (≥ 50%) or complete disappearance of tumors. As the responses can be detected within weeks of treatment, and their durability can be tested for an additional few weeks, the availability of a surrogate end-point of tumor shrinkage often reduces the duration of chemotherapy trials. Although there is little overall correlation of these responses to patient survival, in the case of most solid cancers of adults[18], these responses have come to define how cancer trials are measured. A shrinking tumor may cast a powerful but illusory spell. If one were to apply the same criterion of a response to immunotherapy studies, one runs a real risk of missing the show. Our experience of immunotherapy is demonstrating that it does not usually melt tumors. Its pace is slow and measured, and even when it does result in the shrinkage of human tumors, it occurs over a prolonged period of time. Hence it would appear that chemotherapy may have relied unduly on the response rates and that immunotherapists are well advised to be wary of such reliance. Conversely, although stabilization of disease (stabilization of tumor growth or a 10% reduction in tumor size) is not a valid response category for chemotherapy trials, it may correlate with prolonged survival in immunotherapy. The lack of a surrogate marker for anticancer activity leaves us in a difficult position where all trials must be measured solely on basis of long-term efficacy studies in cancer patients. Ironically and reassuringly, it is precisely this reliance on cumbersome and time-consuming tumor rejection assays *in vivo* in rats and mice that led us to discover the immunogenicity of heat shock protein–peptide complexes in the first place[5,6].

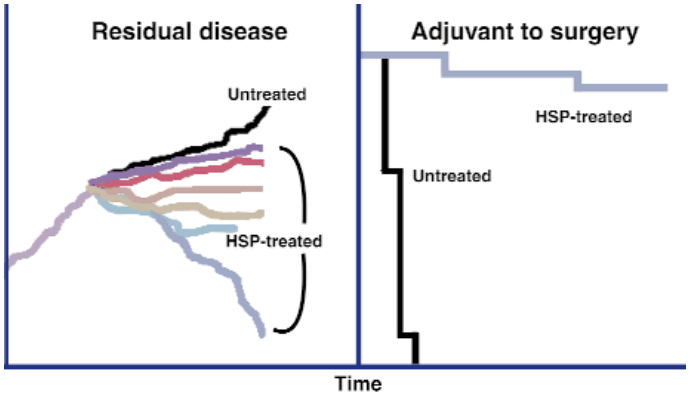

Figure 1. Effect of treatment with tumor-derived heat shock protein–peptide complexes on cancers of mice. (**a**) When mice with large, visible tumors (that is, with residual disease) are treated with heat shock protein (HSP) preparations, the tumors typically show slowed growth, or stabilization of tumor size. Rarely, they show tumor shrinkage. (**b**) When mice that have had surgery with curative intent, and which are apparently disease free but harbor micrometastases, are treated with heat shock protein preparations as an adjuvant to surgery, an overwhelming majority of them are cured and remain disease-free permanently[10].

A question of potency

The second question raised by our attempts to test the heat shock proteins as an approach to treatment of human cancers is how to define the vaccine. The vaccine consists of heat shock protein–peptide complexes isolated from a patient's tumor. It contains a heat shock protein polypeptide chain that chaperones a wide array of undefined antigens, and which is unique to the given tumor. The isolated heat shock protein–peptide complexes are a repository for possibly the entire antigenic repertoire of a tumor, without the need for antigenic definition, and in this is contained the uniqueness and power of the heat shock protein approach. It also poses a unique hurdle. How does one determine that the vaccine being injected into a patient contains those antigens? Such an autologous protein vaccine has never been used in medicine and hence the regulatory rules that govern clinical trials, and demand evidence of "potency" (activity of an agent) have little precedent to guide them. Fortunately, heat shock proteins have biological activities other than chaperoning antigenic peptides. They can mediate maturation of dendritic cells and can stimulate them, and other antigen presenting cells, to secrete cytokines[19–21]. These attributes may be used to assess the potency of the heat shock protein vaccines, even though they do not address the issue of antigenic content. For all practical purposes, the latter issue cannot be addressed with regard to the heat shock protein approach or other autologous approaches that rely on the entire antigenic repertoire of a cancer.

Search for dose, route, regimen

The next question is of dose, and here too there is scant guiding precedent. Traditionally dose has been estimated by starting with the lowest possible dose and working up to a maximal tolerated dose, as guided by animal experiments. The doses deemed effective in animals are transformed into human doses based on calculations of body weight or surface area. Efficacy is then tested on doses lower than the maximum tolerated dose. For the heat shock protein trials this question was particularly sensitive. The quantities of heat shock proteins that can be obtained from human cancers are inherently limited; if larger doses were needed, the approach would be impractical in humans. Hence it was a matter of urgency to resolve the dose question. Fortunately, the answer was both immunologically valid and practically reassuring.

I reasoned that, in contrast to chemotherapy, antibiotics and other drugs, there was no need to maintain any particular blood concentrations of the heat shock proteins. They interact with antigen presenting cells at the site of injection through a receptor[2,4]. Then the antigen presenting cells travel to lymph nodes where they stimulate the naïve T cells. The phenomenon is localized in mice and humans alike and hence we should not have to inject any larger quantities in humans than in mice, or, at the very least, the optimum doses in mice and humans should lie within similar ranges. This thought was consistent with our observations that although mice and rats differ considerably in their total body weight (>20-fold difference), we did not need any larger quantities of heat shock proteins to immunize rats than we did for mice[5,6]. Although this clarified the idea in principle, the actual doses remained to be determined. We started with 25 μg/injection and ranged up and down between 2.5–100 μg/injection. Our preliminary results suggest that we are within an active dose range[7–9].

Questions of route are inextricably linked to those of dose, and having arrived at an answer to the latter we were provided with an opportunity to address the former. Randomized studies in which the

same dose was delivered through different routes have been conducted and are resolving this issue. With respect to regimen, the protocol used so successfully in mice[10] is now being used in patients: patients with existing residual tumors are being immunized as long as the vaccine supply lasts, whereas patients who are rendered apparently disease-free by surgery receive only a limited number of vaccines after surgery. The immunological basis of the need for continued immunization in patients who have existing residual tumors is not clear, as immunization with heat shock protein–peptide complexes does elicit memory responses[22]. However, our collective experience with therapeutic vaccines (as opposed to prophylactic ones) is still quite limited and new ideas are bound to emerge. The suggestion that a signal is required to activate antigen presenting cells may be pertinent in this regard[23].

A key area of ongoing development for immunotherapy with heat shock proteins involves the quantity of tumor tissue necessary to obtain enough heat shock protein for immunization. Typically, one requires approximately 250 μg heat shock protein preparation for ten immunizations. Depending upon the tumor type and recovery, this may be obtained from 2–5 g of tumor tissue. This quantity of tumor tissue is easily obtained from renal cancers, melanomas and colon carcinomas but less easily from pancreatic cancers and some other tumors. Hence, we are in the process of developing methods through which the transcriptome of a tumor biopsy can be expressed in a human cell line that then provides the heat shock protein–peptide complexes specific for that tumor. This process will render obsolete questions of quantity of starting tumor material, and of the yield of heat shock protein preparations from tumor tissue.

The majority of completed and ongoing trials have been done using gp96. This is simply because gp96 was the first heat shock protein immunogen we discovered and more work has been done, therefore, with gp96 compared to hsp90, hsp70 or calreticulin. New heat shock protein immunogens, such as hsp110 and grp170, have been identified in the meantime[24] and, depending on the mechanism through which each heat shock protein elicits immunity to chaperoned peptides, combinations of heat shock protein preparations from individual tumors may further augment the clinical activity.

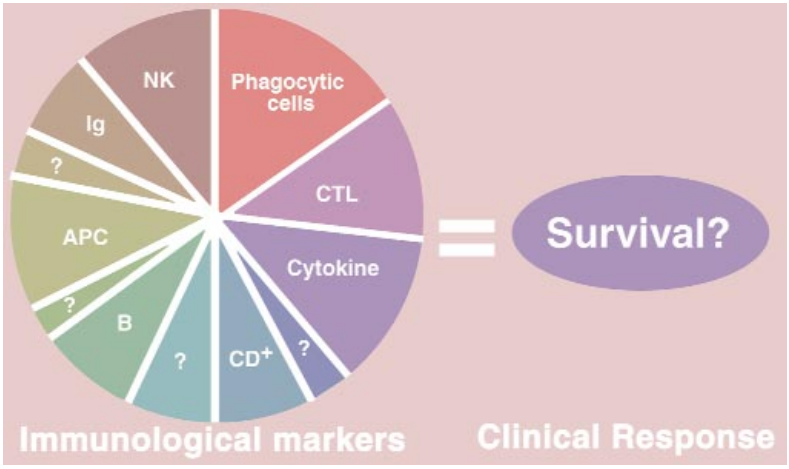

Figure 2. Although an array of immunological markers have been examined as potential surrogate markers of antitumor activity in humans and in mice, none have been found suitable. (NK, natural killer cells; CTL, cytolytic T cells; $CD4^+$, $CD4^+$ T cells; B, B cells; APC, antigen presenting cells; Ig, immunglobulins.)

Listening to the mice

Randomized Phase III trials alone will help us to determine how the heat shock protein approach fares with the "unforgiving yardstick of clinical trials" (L. J. Old, opening comments at the Cancer Vaccine Conference, New York, 1998). Until then, one must simply persevere. The basic scientific observations regarding the immunogenicity of heat shock protein–peptide complexes, once deemed heretical, have now entered mainstream immunology, and the whiff of early clinical data is promising and appetizing across the spectrum of cancers where patients have been immunized. This point in time may be, therefore, particularly suitable for contemplating what we have learnt already.

I will summarize some lessons that we have learned along the way. The first is that undue reliance on "responses" may not be a good idea. Stable disease is not acknowledged as a real response in chemotherapy. However, in immunotherapy trials, it may be a significant category of response, as it may translate better into prolonged survival. Secondly, despite extensive work over the years, there are thus far no real surrogate markers for cancer immunity (**Fig. 2**). Although it is incumbent on us to search for them and to monitor patients diligently, the only end-points of any value today are the clinical end-points. Third, the classical drug development pattern of Phase I, II and III trials is not necessarily a good idea for cancer immunotherapy. Although safety must always be of paramount importance, one should look for clinical end-points early and randomize trials early. It would be useful to enter randomized Phase III trials as soon as a reasonable sense of active dose is obtained, that is, much earlier than is traditionally done.

The final lesson may be the most important: perhaps mice do tell the truth after all. All the early indications tell us that the limited clinical data we have are not in disagreement with the mouse data[7–10]. I have heard many a veteran of the battles of drug development say, with a practiced sigh, that if it were as easy to cure human tumors as it is to cure mouse tumors, the cancer problem would have long disappeared. This cynical view is uninformed by data. It is extremely difficult to cure mouse tumors, as anyone who has attempted to do so knows. If one scans the published literature for treatment of pre-existing established murine cancers, the number of papers can be counted on fingers of one hand without reaching the limit of the counting tool. When approaches that have been used successfully in mice for prophylaxis, for treating tumors established for anything from a few hours to less than a week before treatment, or for treating human tumor xenografts in nude mice fail in clinical trials it is proclaimed that mouse data do not extrapolate to humans. This is inappropriate.

The human cancers that we aim to treat are well established and have taken their time getting there. They have had the opportunity to influence their microenvironments and the larger immunological environment. The mouse models that are used to justify clinical trials must show some semblance to the human disease to be credible. It is entirely possible that some mouse data shall not extrapolate to humans, but this case has not been made with respect to vaccination. Should the case be made successfully, it would be profitable, revealing new immunological principles that would be quite useful in translation between the species. In view of the lack of such a case based on real data, the cynicism is unfounded and is damaging to the spirit

of scientific enquiry. Vaccination is one of the few true success stories of modern medicine and its principles have translated well and easily from cows to humans, at least in the case of infectious diseases. As of today, there is little reason to believe that they shall not do so in the case of cancer.

Let us consider critically the possible reasons why these principles may not translate as well in case of cancers. First, infectious agents are clearly of foreign origin, whereas cancers are self-entities. Although this is superficially true, it has now been shown that human cancers carry a mutational (nonself) repertoire[25] of immunogenic potential[26]. Also, one can prophylactically immunize against cancers as effectively as against an infectious agent. Second, there is the argument that cancers harbor immunosuppressive agents that thwart the immunization process. In response to this, one must begin with the recognition that cancer patients do not generally suffer from opportunistic infections, or other symptoms of acquired immune deficiency, neither are they generally immunocompromised. The immunological suppression associated with cancers is cancer-specific and may be limited to a response to the dominant antigens. Immunization approaches that use a broad repertoire of antigens rather than a selection of common and shared antigens are likely to, and in mouse studies are demonstrably able to, pierce this barrier to successful immunotherapy. Finally, success in the case of infectious disease has a prophylactic setting, whereas the challenge of cancer lies in the domain of treating pre-existing disease.

This is an interesting and valid difference. We have as little experience with immunotherapy of pre-existing infections as with immunotherapy of pre-existing cancers. We do not understand clearly the reasons that make therapy profoundly different from prophylaxis. However, emergence of the very first approaches, such as the heat shock protein approach, that can treat pre-existing cancers in mice sheds light on these differences, and can begin to guide us towards immunotherapy of not only human cancers but also of human infectious diseases. In this way, cancer immunology may begin to repay its debt to infection immunology.

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