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The new vaccines: building viruses that elicit antitumor immunity

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Abstract

Whereas cancer cells are poor immunogens, some viruses are capable of eliciting powerful and lifelong immunity. Recombinant viruses and plasmid DNA encoding tumor-associated antigens can elicit powerful and specific immune responses that can be enhanced by the use of cytokines and costimulatory molecules. These immune responses have destroyed growing tumor cells in experimental animal models. For the first time, immunotherapeutic strategies that employ recombinant viruses are being tested in clinical trials with cancer patients.

Introduction

Cancer cells are notoriously poor immunogens. For over a century, immunotherapists have attempted to increase the immunogenicity of transformed cells, beginning with Coley's injection of live cultures of *Streptococci erysipelas* into growing tumor nodules [1]. Since these early efforts, tumor cells have been irradiated, admixed with a number of adjuvants including *Corynebacterium parvum* and Bacille Calmette-Guérin (BCG), and infected with viruses to create 'oncolysates'. More recently, we and others have attempted to 'gene-modify' tumor cells by inserting DNA sequences encoding a variety of immunomodulatory molecules.

An entirely different and potentially more effective immunotherapeutic strategy employs recombinant and synthetic forms of tumor-associated antigens (TAAs) that are recognized by immune cells. This approach is possible because TAAs have now been cloned, and their identities are reviewed in this section and elsewhere [2-4]. As a result, instead of using tumor ceils as immunogens, peptides can be synthesized whose sequences correspond to epitopes of TAAs that are recognized by T lymphocytes. Alternatively, genes encoding TAAs are inserted into recombinant vectors.

The impetus for using synthetic and recombinant immunogens comes from the observation that attempts to alter tumor cells in order to increase immune recognition of TAAs are largely unsuccessful, while many attenuated, killed or recombinant viruses or viral extracts can elicit powerful, specific and lifelong immunity. For example, the threat of smallpox, rabies, polio, hepatitis and even influenza has been eliminated or attenuated. Thus, the specific question that we address in this review of the current literature is whether synthetic or recombinant immunogens can be constructed that are capable of eliciting more powerful antitumor immunity than tumor cells. If so, can these immunogens be used in the treatment of established cancer?

Developing experimental animal models

To address the question of whether some viruses are intrinsically better immunogens than tumor cells, we and others have studied the immune responses to model antigens expressed by

Note added in proof All mg quoted in reference [27] should be read asμg.

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viruses and compared them with the responses elicited when these same antigens are expressed by tumor cells.

A broad array of animal data suggests that viral immunogens better tumor cells by any number of measures. For example, investigators have studied immune reactivities to experimental antigens such as β-galactosidase (β-gal) from *Escherichia coli*, chicken ovalbumin (OVA), and nucleoprotein (NP) from vesicular stomatitis virus (VSV). Genes encoding these antigens have been inserted into tumor cells and cloned into viral DNA (Table 1). The growth rates and lethality of tumor cells transfected with these experimental antigens is often unchanged. Additionally, the cellular immune responses against antigens expressed by growing tumor cells are very weak, if measurable. In contrast, the expression of these same experimental antigens by any number of different recombinant viral vectors elicits powerful and specific cell-mediated immune reactivities when measured in proliferation, cytokine release and microcytotoxicity assays [5•].

The most important immunological finding is that mice bearing model antigen (Ag) expressing tumors can be treated successfully when they are inoculated with viruses capable of mediating the expression of the same model antigens. For example, pulmonary metastasis can be reduced and survival can be extended in mice bearing the CT26 β-gal expressing tumor (CT26.CL25) if they are infected with a recombinant fowlpox virus that also mediates the expression of β-gal [5•]. Such an experimental result has clear implications for the design of new immunotherapeutic strategies in the clinic.

Choosing a vector

The ideal vector for the construction of a recombinant virus-based anticancer vaccine does not exist. But like the concept of a perfect circle we can imagine its qualities. The most important quality would be that it is safe and nononcogenic. In addition, the ideal vector would be nonintegrating, nonreplicating, nonimmunosuppressive, easily engineered and genetically stable.

The ultimate vector would mediate the expression of a desired amount of heterologous protein with the kinetics of expression that could be maximized by the immunotherapist. For this purpose, vector-mediated transcription may have advantages over transcription that is mediated entirely by the unaltered transcriptional machinery of the host cell. Translation, however, might be best carried out by the machinery of the host cell, as this would allow the intracellular targeting of heterologous protein (for example to the endoplasmic reticulum, the compartment for peptide loading or the cell surface).

The perfect vector might have an assignable tropism. For example, the vector could be designed to preferentially infect ‘professional’ APCs like dendritic cells, or alternatively for tumor cells. Finally, the perfect vector would be capable of expressing multiple different heterologous sequences such as TAAs together with cytokines and costimulatory molecules. Although the ideal vector may not exist, a number of vectors that possess many useful qualities have been constructed and characterized (Table 2). Recombinant forms of each of these vectors have been shown to be capable of eliciting antitumor reactivities.

Antigen processing and the optimization of TAA structure

T lymphocytes recognize peptides that are generally processed fragments of proteins that must be denatured and cleaved and transported to specialized intracellular compartments. In some cases these peptides are trimmed again prior to their presentation at the cell surface where they are complexed with MHC class I or class II molecules. Recombinant vaccines can be designed

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to optimize the presentation of immunogen to T cells through an understanding of the cellular and molecular biology of antigen processing.

Two recently published strategies illustrate this point. Firstly, to explore the extent to which $CD8^+$ T lymphocyte induction *in vivo* was limited by proteolysis or peptide transport into the endoplasmic reticulum (ER), recombinant vaccinia viruses were constructed that contained minigenes encoding antigenic peptides (bypassing the need for proteolysis), or these peptides with a amino-terminal ER insertion sequence (bypassing the requirements for both proteolysis and transport). In every circumstance examined, targeting peptides to the ER never diminished, and in some cases greatly enhanced, the $CD8^+$ cell immune response [6,7•,8,9]. Secondly, to optimize the presentation of peptide antigens for recognition by $CD4^+$ T lymphocytes investigators have rerouted antigens to the MHC class II pathway. This was accomplished by creating vaccinia viruses that mediated the expression of human papillomavirus E7 protein fused to a lysosomal-associated membrane protein, (LAMP)-1. Immunogenicity was enhanced as measured by lymphoproliferative activity, antibody titers, cytotoxicity assays and antitumor activity [10••,11•].

The right stuff: defining adjuvanticity

The term adjuvant (from the Latin *adiuvo*, meaning ‘to help or assist’) generally refers to ‘vehicles’ that are used to enhance antigenicity. An example of a powerful adjuvant that has been used successfully in innumerable animal studies is Freund’s complete adjuvant (CFA), a mixture of water, oil and killed mycobacteria. Whereas the formulation of adjuvants was entirely empirical in the past, a deeper understanding of the mechanisms of immune activation makes their enlightened design a real possibility.

The components of adjuvant function have now come into focus, and it is clear that the expression of TAAs by viruses accomplishes some of the functions of an adjuvant. These functions include altering the extracellular and intracellular trafficking of antigens, providing additional antigenic substrates for specific and nonspecific immune recognition, prolonging the kinetics of antigen exposure (depot function), and augmenting the effective concentration available for immune recognition.

Researchers have identified cytokines and costimulatory molecules that are critical elements of an immune-activating microenvironment. We and others have found that some of these molecules can enhance the ability of recombinant and synthetic immunogens to mediate the regression of an established tumor. In particular, the administration of the T lymphocyte growth and differentiation factors interleukin (IL)-2 [12•] and IL-12 [13] have profound adjuvant activity. Contrary to much of the published literature, IL-10 also can facilitate the activation of powerful cellular immune responses elicited by recombinant viral vaccines (H Kaufman, Rao JB, SA Rosenberg, NP Restifo, unpublished data).

Use of ‘professional’ APCs as whole cell adjuvants

In order to manipulate more precisely the process of antigen presentation, scientists have made substantial efforts to understand the biology of professional APCs, including dendritic cells, and to use these cells as adjuvants for immunization [14••,15•-17•,18,19,20•]. APCs originating from bone marrow, spleen, or peripheral blood can be differentiated *ex vivo* using a variety of cytokine formulations. Expression of the relevant TAA is then conferred upon these cells by coincubation with the relevant peptide epitope, by transduction of an undifferentiated precursor population with a retrovirus containing the gene for the TAA, or by infection of a differentiated population of dendritic cells with a virus capable of mediating the expression of TAAs (Marincola F, Hwu P, SA Rosenberg, NP Restifo, unpublished data). These antigen-expressing ‘professional’ APCs can then be used *in vitro* to prime or restimulate

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antitumor T lymphocytes or *in vivo* as whole cell adjuvants to immunization with synthetic and recombinant anticancer vaccines.

‘Releasing the brakes’ on an antitumor immune response

In addition to supplying positive costimulatory signals, eliminating negative regulatory signals may also enhance antitumor immune responses. The phenomenon of tumor-induced immunosuppression has been highly controversial: it was seen in some tumor models but not in others and the guilty suppressive factors have not been convincingly identified on a cellular or molecular level.

While the efforts to identify the elements responsible for tumor-induced immunosuppression are too diverse and multifaceted to summarize here, a few examples could serve to illustrate mechanisms potentially useful in the design of recombinant anticancer vaccines. One provocative example is illustrated by signaling through CTLA-4 (a cell surface molecule found on activated T cells), which, if abrogated, can enhance antitumor reactivity [21,22•]. Another hint of how the inhibition of negative regulatory signals could enhance the T cell response is a potential blockade of the propensity of activated T cells to undergo apoptosis when re-exposed to the same antigen. The interaction of Fas and Fas ligand and of tumor necrosis factor (TNF) and the p75 TNF receptor can mediate the apoptosis of T lymphocytes [23•,24]. Thus, blockade of these pathways could potentially be used to ‘release the brakes’ on an antitumor immune response [25].

Building new vectors

Genetically engineering recombinant viral immunogens to enable them to mediate the expression of immunomodulatory molecules together with a TAA can greatly enhance their function. Examples include the coexpression antigen β-gal, and B7-1 [26] or IL-2 [12•]. Viruses can also be constructed that encode combinations of immunomodulatory molecules like B7-1 and IL-12 together with TAAs. This combination has proven effective when the IL-12 is provided exogenously [27]. Such an elaborate construction would be difficult or impossible to build using a single plasmid recombined into a single site of the virus genome. Instead, a vector was constructed which employs a viral ‘cassette’ system, in which three different regions of the recombinant viral genome are used: viral protein 37 for the B7-1 costimulatory molecule; the thymidine kinase locus for the model antigen, β-gal; and the hemagglutinin locus for both chains of the heterodimeric IL-12 molecule (M Carroll, personal communication).

Obstacles to successful immunotherapy using recombinant vaccines

Despite our great enthusiasm for development of anticancer vaccines, there are several major experimental issues that are not yet adequately addressed in animal models [28]. The first concerns the duration of tumors in humans. Whereas tumor deposits may exist for years in humans before they are treated, or even detected, the time course studied in mice is generally measured in weeks and sometimes even in days. The longer kinetics of the tumor-bearing state could increase the heterogeneity of the tumor cells, resulting in cell to cell differences that include antigen expression and antigen processing and presenting efficiency. Human tumor cells can escape immune recognition by a number of mechanisms, including loss of β_2-microglobulin [29], downregulation or loss of the expression of particular HLA class I loci [30], and downregulation, mutation or deletion of the proteasome component molecules latent membrane proteins -2 and -7 as well as of transporters associated with antigen processing [31].

Prior chemotherapy or radiotherapy could complicate problems related to the mutability of tumor cells. Such mutability can result in powerfully resistant tumors when the numbers of

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tumor cells are counted in trillions. Furthermore, when tumor weight is measured in kilograms rather than in grams or milligrams, issues of peripheral tolerance as well as other forms of specific and nonspecific immunosuppression could be qualitatively different.

Another factor that is difficult to model in animals is anamnestic responses to recombinant viral vectors that are natural pathogens in people, but not in mice. We have already seen in the clinic (SA Rosenberg *et al.*, unpublished data) that most patients have high circulating levels of neutralizing antibodies to the adenoviral vectors we employ. The same is true for recombinant vaccinia virus immunogens, where the vast majority of patients being immunized received vaccinia as children during the effort by the World Health Organization to eradicate smallpox.

Tolerance and the antitumor immune response

For tumor immunologists, the concept of 'self versus non-self' as the central tenet of modern immunology is a concept that has needed revision in recent years. On one hand, large antigens that have little homology with known mammalian proteins, like β-gal from *E. coli*, or chicken OVA, or NP from VSV, can be transduced into tumor cells without significantly affecting their growth rate or lethality, and without attracting any measurable immune responses. Thus, the immune system does not simply reject what is non-self (i.e. foreign). On the other hand, cloning TAAs recognized by T lymphocytes and that are expressed by human melanoma cells did not reveal that these TAAs contained the myriad mutations characteristic of the transformed genome. Instead, these TAAs were generally nonmutated tissue differentiation antigens. Hence, the immune system can indeed recognize components of 'self'.

The expression of melanoma TAAs by normal melanocytes has raised new immunological issues, including those of central and peripheral tolerance and the induction of immune responses to these 'self' TAAs. To develop experimental mouse tumor systems that more accurately model human cancers in which TAAs are tissue differentiation antigens, our group and others are currently studying immune reactivities to the murine homologues of the human tumor rejection antigens MART-1, gp100, tyrosinase and tyrosinase related proteins (TRP)-1 and -2. Interesting consequences of such 'self' reactivity are vitiligo in patients receiving the T cell growth factor IL-2 [32•] and coat color changes (black to white) in mice treated with gp75 (brown locus) antibodies [33••].

The situation could be different in cases where viruses are believed to be causally involved in the process of cellular transformation, such as cervical cancer (human papillomavirus), liver cancer (hepatitis B), and nasopharyngeal carcinoma and Hodgkin's, Burkitt's, T cell and immunoblastic lymphomas (Epstein–Barr virus). Because viruses appear to be responsible for a minority of cancer deaths, however, issues of central and peripheral tolerance are likely to continue to bedevil cancer immunotherapists.

Conclusion

Recombinant vectors can be constructed that are capable of mediating the expression of TAAs. Infection of tumor-bearing mice with these viruses can result in powerful antitumor immune responses and the resulting reduction of tumor burden is matched by prolongation of survival. As we incrementally improve the function of recombinant anticancer vaccines in the laboratory, attempts are being made to translate these findings to the clinic [34•]. Clinical trials have now been initiated and we all anxiously await their results.

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Abbreviations

APC
: antigen-presenting cell

β-gal
: β-galactosidase

ER
: endoplasmic reticulum

IL
: interleukin

OVA
: ovalbumin

TAA
: tumor-associated antigen

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Table 1

Selected examples of mouse models used in studies of recombinant and synthetic anticancer vaccines

Tumor	Antigen	References
CT26	β-galactosidase	[5•,35]
Clone 10	Ovalbumin	[36]
E22	Ovalbumin	[20•]
MC38	Carcinoembryonic antigen	[37]
A20	Mutant Ras	[38,39•]
P815	P815A	[8,40•]
Lewis lung	Mutated connexin 37	[41,42]
CT26	gp70 from murine leukemia virus	[43••]
6132A	Mutated ribosomal protein L9	[44]
B16	Murine homologues of melanoma TAAs	[45]

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Table 2

Selected recent examples of recombinant vectors as anticancer vaccines

Vector	References
Vaccinia	[46]
Modified vaccinia virus Ankara	[47]
Fowlpox	[5•]
Canarypox	[48]
Adenovirus	[49,50]
Influenza	(a)
Poliovirus	[51]
Sindbis	[52]
Nonviral constructs:	
Naked DNA	[53]
Gene gun	[54•]

(a) NP Restifo, DR Surman, P Palese, SA Rosenberg, A Garcia-Sastre, unpublished data.