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Cancer Immunity, Vol. 3, p. 4 (18 June 2003)

Hypothesis: Controlled necrosis as a tool for immunotherapy of human cancerPramod K. Srivastava 

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Keywords: cancer, immunotherapy, necrosis

Considerations

The number of ways in which cells die is broadly characterized into apoptosis and necrosis (see [1](#) for a more nuanced and accurate view). Apoptosis refers to programmed cell death, where exposure of a cell to events such as radiation, certain cytokines, or drugs leads to activation of a specific cascade of events that end in DNA fragmentation and a 'clean' death. The contents of the cell are not released into the external milieu but get packaged into apoptotic bodies which may be scavenged by neighboring phagocytes. End of story. Necrotic death on the other hand may result from unplanned events, such as lytic infection by a virus, loss of membrane permeability, mechanical tearing of a cell, etc. Such cell death leads to spilling of the cellular contents into the immediate milieu and is thus 'not clean'.

A number of recent studies ([2](#), [3](#), [4](#)) have shown that in addition to not being a clean death, necrotic cell death is also not a quiet death. It is heard by the immune system which responds to it through both of its major arms, the innate and the adaptive. Dendritic cells (DCs) and related cells, such as macrophages, are the key components of both arms of the immune system ([5](#)) and are the cell types that respond to necrotic cell death. DCs bear receptors for the abundant cellular contents released by necrotic cell death e.g. heat shock proteins (HSPs) ([6](#), [7](#)) and DNA ([8](#)) and respond to them in multiple ways. Engagement of the HSP receptors, and presumably of the DNA receptors on macrophages and DCs, signals the primordial NF-kappaB pathway so important in biology ([9](#)). Such engagement stimulates macrophages to secrete cytokines and chemokines, and DCs to show changes characteristic of maturation and migration to the draining lymph nodes ([4](#), [10](#)). The HSPs mediate another powerful reaction that engages the adaptive immune system. The major HSPs have been shown to be associated *in vivo* with a large repertoire of cellular peptides, including antigenic peptides generated by degradation of the proteins within a cell ([11](#)). When necrotic death leads to the release of these HSP-peptide complexes, they are taken up by the DCs through the HSP receptors mentioned earlier. The peptides chaperoned by the DCs get re-presented by the DCs by their MHC class I and II molecules which in turn stimulate the CD8+ and CD4+ T cells ([11](#)). Thus necrotic cell death engages the innate *and* adaptive components of the immune system. This mode of cell death can be utilized for the therapy of human cancer.

HSPs constitute a, if not the, major component of the innate and adaptive immuno-stimulatory activity of necrotic cells. Indeed, the HSPs (or more accurately, HSP-peptide complexes) purified from cancers have proven their

cancer therapeutic activity in multiple studies with primary and metastatic cancers in mice, rats and frogs ([12](#), [13](#)) and are currently in multiple randomized Phase III clinical trials for the therapy of human cancer. The Phase I and II studies have yielded preliminary evidence of the immunological and clinical activity of HSP preparations, in addition to their safety and feasibility ([13](#), [14](#), [15](#), [16](#), [17](#), [18](#)).

In these clinical trials, HSP-peptide complexes are isolated from tumor tissues obtained by surgery or biopsy and are used for the therapy of the patient from whom the tumor sample was originally obtained. This practice raises the following question: If cancer-derived HSP-peptide complexes can mediate therapy of the very cancers from which they are isolated, why can they not mediate the same effect *in situ*, particularly as necrosis to some degree is a common aspect of growing tumors? In other words, why do the cancers not self-immunize if the immuno-stimulatory activity that can be used to treat them resides within them? While one cannot rule out that such self-immunization indeed happens in the case of tumors we never see, it clearly does not occur in a significant number of instances that we do see. The answer to this paradox lies in at least two parts, one involving quantity and the other location.

First, the quantity. We have demonstrated earlier that the immunizing activity of HSP-peptide complexes is highly dose-restricted ([19](#), [20](#)). A sub-optimal quantity does not immunize, an optimal quantity immunizes, and a supra-optimal quantity not only does not immunize but actually suppresses the immune response. Thus, in order for a host to effectively self-immunize against a growing tumor, it would have to control the extent of necrosis in some manner. Unfortunately and obviously, this process is not under voluntary control.

Let us now visit the question of location. The HSP-peptide therapeutic vaccines are given to mice and humans through immunization at a healthy site in the skin. We have observed that immunization of mice with optimal quantities of HSP-peptide complexes at the tumor site is ineffective ([21](#)). This observation is consistent with the large body of literature showing that the tumor microenvironment is not particularly immuno-stimulatory and that the tumor-resident DCs may be functionally deficient ([22](#)).

Other factors that may be responsible for the lack of self-immunization through necrotic tumor lysates may exist; however, the two factors of quantity and location of necrosis, identified here, are important. In the instances where successful self-immunization does occur (discussed previously), one may imagine it to be happening in very small tumors that happen to generate optimal quantities of necrosis and do not create the immuno-subversive microenvironment characteristic of larger tumors.

The hypothesis

The hypothesis now becomes self-evident. If we can generate a controlled amount of necrosis artificially, and if we can immunize the patient with this lysate, it might be a relatively simple way to build on the cell biological and immunological concepts discussed in the beginning of this article, concepts whose proof of principle exists in animal studies and appears to be emerging through human trials ([12](#), [13](#), [14](#), [15](#), [16](#), [17](#), [18](#)). Thus, a surgical instrument(s), that:

- a. can be inserted into a tumor,
- b. can excise a tumor core of measured volume of a defined diameter,
- c. permits the introduction of a fluid such as sterile saline into the chamber in which the tumor core has been placed,
- d. permits the 'blending' (as in the homogenization of tissues) of the tumor core while still

- within this chamber, perhaps through a motorized mechanism, and
- e. scarifies the skin of the patient at a tumor-adjacent or distant site and/or immunizes with the lysate through an attachment or other mechanical device,

could effect such *in situ* immunization.

Individual components of this instrument already exist. Components (a) and (b) are routinely used to obtain core biopsies, (c) and (d) would require feasible and economical modifications of the needles used for core biopsy, and (e) used to be the method of immunization of choice in the early days of vaccination against smallpox. Component(s) functionally equivalent to these can be easily imagined.

The treatment and this device could be augmented through several means. The tumors could be subjected to local hyperthermia or other means of radiation that may be deemed or determined to be capable of enhancing the expression of HSPs. Additional components including cytokines such as granulocyte macrophage colony stimulating factor, adjuvants such as alpha₂-macroglobulin, or agents that neutralize immunosuppressive moieties, could be added to the lysate to enhance their immunogenicity.

Testing the hypothesis

The hypothesis may be tested with animal tumors with pre-existing cancers at various stages as follows. The optimal quantity of tumor lysate that can mediate immunotherapy can be determined experimentally. Based on our previous experience with HSP-based immunotherapy, this quantity is expected to be the same for most if not all tumors, and similar across species. Before developing a prototype of the instrument imagined here, the means of delivering the lysate can be tested by means of delivery already available, e.g. through syringes, scarification, and mini-pumps. Should the animal results conform to the hypothesis, human trials may begin following the traditional route of drug development.

One may imagine several reasons why the intervention suggested here may not work. The history of human cancer immunotherapy is replete with unsuccessful efforts to treat cancer patients with tumor lysates of many kinds (see [23](#) for review). However, a perusal of that vast effort does not show requisite attention, or indeed any attention, to the crucial question of quantity of lysate, as posited here. Nonetheless, the two factors of quantity and location may not be the only reasons for lack of self-immunization. Other hurdles may exist. The immunosuppressive factors present in tumor lysates (such as TGF-beta) may mask the immunogenicity of the HSP-peptide complexes present in lysates. There might exist an unknown variability in the quantity of lysate required to immunize effectively. The quantity of lysate may be more difficult to control than imagined. Nonetheless, the hypothesis is based on cell biological and immunological principles that have been extensively verified and is eminently testable. Experimental testing shall either confirm the hypothesis or reveal other factors that may require additional consideration, and lead to further refinement of the hypothesis.

Abbreviations

HSP, heat shock proteins

Acknowledgements

This article was conceived, and partially written, during a boat trip on the Thames organized by the International Society for Cancer Gene Therapy. I acknowledge their contribution in having provided the milieu. I also thank Richard Zeff of my university for his critical review of the manuscript. I am the scientific founder of Antigenics, Inc. in which I have a significant financial interest.

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