

Mechanisms of Multimodal Immune Therapy for People with Cancer

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Introduction

It is well recognized that patients who have cancer have multiple defects in their immune signaling and intercellular communication pathways, else a tumor likely would not grow. Improvement in such pathways, in attempt to annihilate such growths and prevent their recurrence, is thus prudent. Current conventional therapy for cancer that has spread to distant sites is inadequate¹ thus necessitating different strategies. Specific immunotherapy is one such strategy that, given the proper circumstances, is quite effective.

Ultimately, lasting eradication of malignancy, as well as prevention of malignancy in the first place, depends solely upon competent immune function. Cyto-reductive strategies, such as radio- or chemotherapy, serve their intended purpose of rapid tumor reduction but are hopeless at creating or even enabling a full endogenous immunological response. Unsurprisingly, then, such therapies diminish self-defense mechanisms leaving the host susceptible not only to infectious disease, but to recurrence and metastasis of their cancer as well.

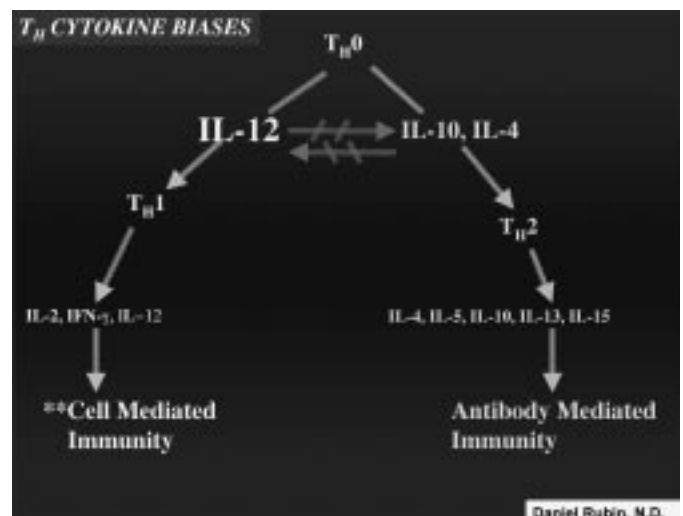
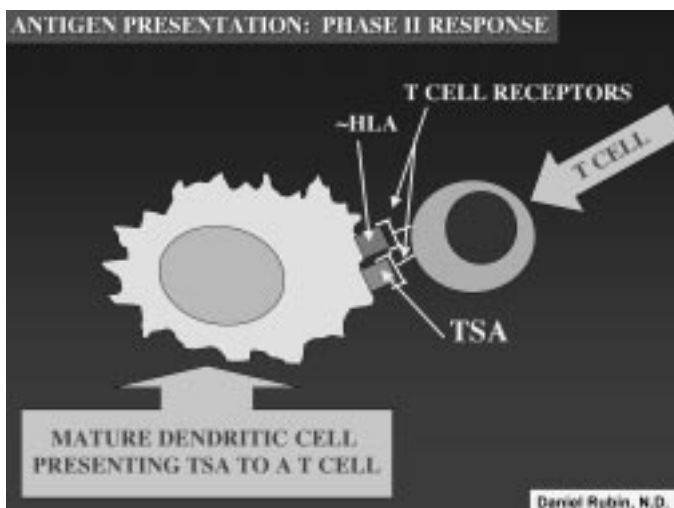
Immunotherapy

Specific immunotherapy entails the use of a combination of immunogenic agents to elicit an appropriate antitumor immune response. In order to achieve such a response, reestablishment of the proper cell mediated immune cascade must be permitted. There are two main arms of the immune system: humoral immunity and cell mediated immunity.² The former is concerned mainly with activation and promotion of the B lymphocyte pathway, ultimately leading to the production and secretion of antibodies against a given target. The latter denotes a T-cell mediated response which eventually leads to the development of a specific

clone of T cells, educated and determined to attack and destroy any cell bearing the target peptide with which it was presented. Presentation refers to the process wherein fully matured dendritic cells that have captured tumor-specific antigen [TSA] process TSA into a form presentable to T cells, in- or not in-association with the human leukocytic antigen. Antibody mediated immunity and cell mediated immunity are both under the control of cytokines. Basically, depending on the endogenous cytokine profile bias, one pathway will be more dominant.²

Antibody mediated immunity is dominant when the phenotype bias are cytokines from T helper type 2 cells (TH_2) (Interleukin [IL]-5, IL-10 and IL-13 for example) and the cell mediated system becomes dominant when the bias is towards a T helper type 1 response (TH_1) (IL-12, IL-2 and interferon [IFN]- γ for example). The diagram below depicts this difference.

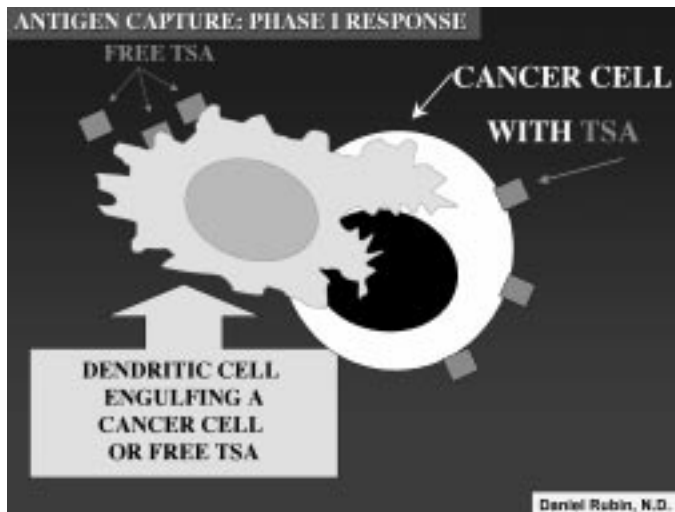
The TH_1 and TH_2 pathways are mutually inhibitory.³ Literally, a switch of the phenotypic bias of the cytokine system can be achieved by altering the circulating cytokine levels.⁴ One immuno-therapeutic strategy is to create an upsurge in the endogenous production of IL-12. Orally administered muramic acid moieties, in particular muramyl polysaccharide glycan complex, exhibit this activity.⁵ Establishment of such bias is important to the success of an antitumor immune therapy.



Once established, monocytes in the body will become converted into immature dendritic cells⁶⁻⁷ that are then able to scavenge both tumor cells as well as freely circulating TSA.

This event is followed by a maturation of the dendritic cell into a professional antigen-presenting cell.⁶⁻⁷ Immature dendritic cells do not present antigen well; on the contrary, matured dendritic cells are potent antigen presenters, thus the entitlement of

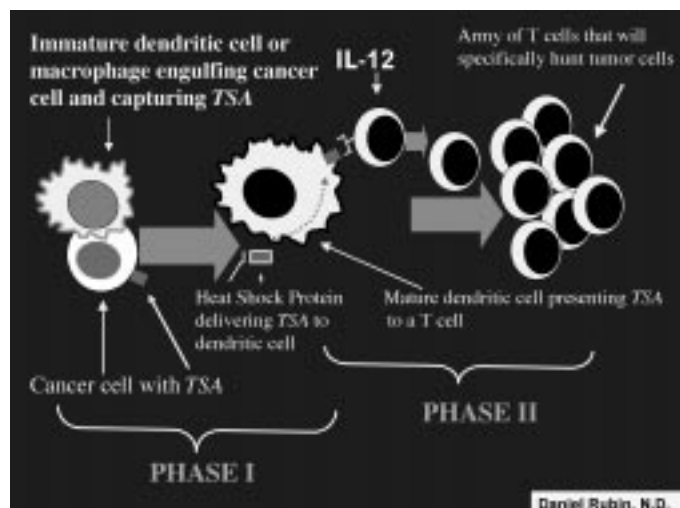
¹Morton DL, Ollila DW, Hsueh, EC, Essner R Gupta RK. Cyto-reductive Surgery and Adjuvant Immunotherapy: A New Management Paradigm for Metastatic Melanoma. *CA Cancer J Clin* 1999;49:101-116. ²Constant SL and Bottomly K. Induction of TH_1 and TH_2 CD4+ cell responses: the alternative approaches. *Annu Rev Immunol* 15:297-322, 1997. ³<http://www.stanford.edu/~smithr/science/apc.html>. ⁴Romano CC, Mendes-Giannini MJ, Duarte AJ, Benard G. IL-12 and neutralization of endogenous IL-10 revert the in vitro antigen-specific cellular immunosuppression of paracoccidioidomycosis patients. *Cytokine* May 7, 2002;18(3):149-57. ⁵Riordan NH, Meng X, Taylor P, Riordan HD. Effects of cell wall extracts of gram positive bacteria (MPGC) on human immunity and tumor growth in animals. Presented at *Comprehensive Cancer Care 2000*, Arlington, Virginia June, 2000. ⁶Romani N, Reider D, Heuer M, Ebner S, Kampgen E, Eibi B, et al. Generation of mature dendritic cells from human blood. An improved method with special regard to clinical applicability. *J Immunol Meth* 196:137-51, 1996. ⁷Reddy A, Sapp M, Feldman M, Subklewe, Bhardwaj N. A monocyte conditioned medium is more effective than defined cytokines in mediating the terminal maturation of human dendritic cells. *Blood* 90:3640-3646, 1997.



“professional.”⁸ Antigen presentation is a crucial step in the advancement of the proper antitumor immune response.

Antigen Presentation

Presentation of antigen (TSA) to a T cell is the second to last step in the cell mediated immune cascade. The last step is a vigorous proliferation of the stimulated T cells into an “army” of T cells all destined towards a common goal: destruction of that cell which bears the formerly-presented TSA. An overall depiction of both phases of these immune events is presented below. Note that without proper function of phase I, the response will not proceed into phase II. Sometimes, it is the lack of a phase II shunt that impairs a full immune attack on the cancer cells. There are two crucial steps in phase I that, when not functioning properly, will inhibit the progression of the immune response: lack of antigen capture and impedance of dendritic cell maturation. The latter is a hallmark of certain types of tumors, for instance, renal cell carcinoma (*kidney cancer*).⁹ In this situation, antigen uptake is not a problem, but maturation of the dendritic cells is inhibited by tumor-derived soluble factors. In the former situation, the problem lies in the initial step of antigen capture. As described in the second illustration, if the dendritophages are unable to uptake antigen, the cell mediated immune cascade will not proceed.



Immunogenicity of Cancer Cells

A main precept for any immune therapy for people with cancer is to make the cancer cells immunogenic (*stimulatory to the immune system*) and subsequently the immune cells responsive to this immunogenicity. Immunogenicity can be induced in several ways, three of which will be focused on here: 1) heat shock protein therapy; 2) angiogenesis and lymphangiogenesis inhibition; and 3) the use of microfractionated doses of traditional chemotherapeutic agents.

Heat shock proteins (HSPs), are small proteins found in cells of all living forms.¹⁰ HSPs are generated by cells under times of stress—heat stress, cold stress or oxygen deprivation. Under these conditions, HSPs are produced by the cells as “repair proteins.” HSPs are able to perform various functions inside the cell, for instance refolding of stress-induced deformation of large proteins, in order for the cell to maintain proper function. Inside a cancer cell, HSPs will combine with abnormally produced proteins or peptides and shuttle them around the cell. Acting then as “garbage collectors” they deliver the abnormal proteins/peptides to other molecules in the cell which then transport them to the surface of the cell, thereby displaying the abnormal protein/peptide complex on the outside of the cell for the immune system to recognize.

Heat Shock Proteins

Recently HSPs have been identified to have broad ranging immune system-related functionality. One author has touted HSPs as the “Swiss Army Knife” of the immune system¹¹ as a real-life exemplification of these effects.

The last slide on this page describes the major effects of HSPs on the immune system. Most importantly, HSPs, can gain access to the interior of a cell. When carrying TSA into a dendritic cell the HSP uses a special gate called, CD91, to enter the cell.¹² Once inside, the TSA is released from the HSP-TSA complex and taken through a series of steps the cell uses to make the TSA recognizable to the rest of the immune system. The sequence beginning at the top of this column illustrates these steps.

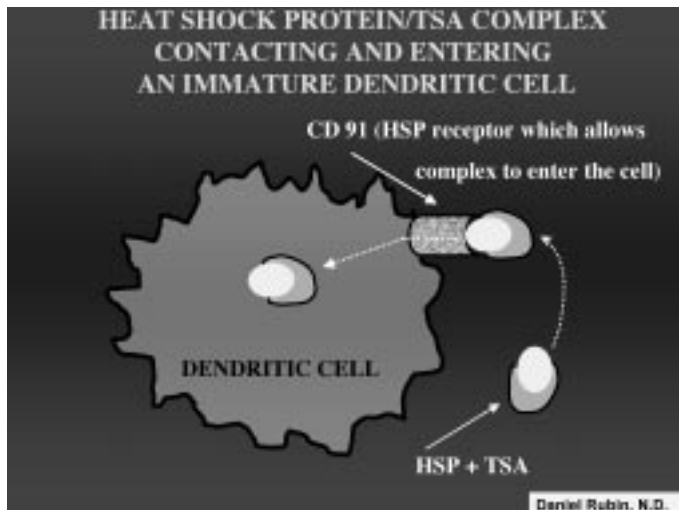
The illustrations at the top of the next page exemplify a third mechanism for loading dendritic cells with TSA. By utilizing HSPs as chaperones of TSA, dendritic cells are spared having to engulf free TSA or the lengthy and energy-expensive task of

HEAT SHOCK PROTEINS

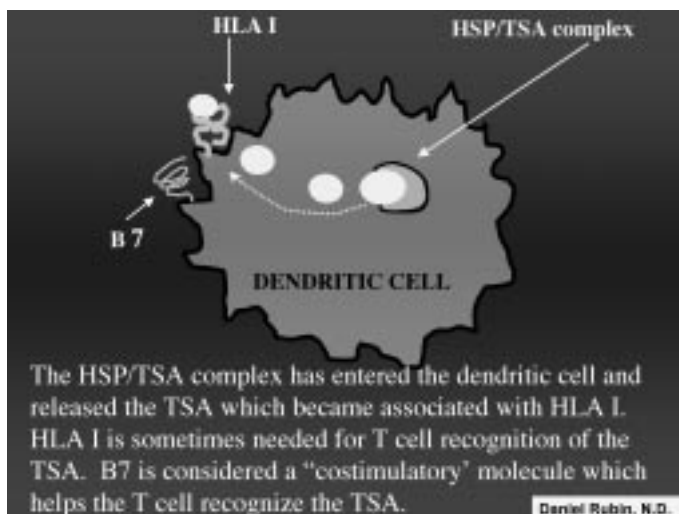
- Bind to antigenic peptides
- HSP-antigenic peptide complexes enter phagocytic cells
- The HSP-chaperoned antigenic peptides prime CD8+ T cells and thus gain access to the MHC I pathway
- 4. HSPs are very efficient: they elicit immune responses in microgram quantities complexed to nanogram quantities of peptide
- CD8+ T cell clones have been developed via stimulation by HSP-chaperoned defined CD8+ epitopes

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⁸<http://www.cat.cc.md.us/~gkaiser/microrlo/unit3/intro/apc/macrodendritic/macdendritic.html>. ⁹Troy AJ, Summers KL, Davidson PJ, Atkinson CH, Hart DN. Minimal recruitment and activation of dendritic cells within renal cell carcinoma. *Clin Cancer Res* 1998 Mar;4(3):585-93. ¹⁰<http://www.antigenics.com/tech/hsp/> ¹¹Srivastava PK, Amato RJ. Heat shock proteins: the ‘Swiss Army Knife’ vaccines against cancers and infectious agents. *Vaccine* 2001 Mar 21;19(17-19):2590-7. ¹²Basu S, Binder RJ, Ramalingam T, Srivastava PK. CD91 is a common receptor for heat shock proteins gp96, hsp90, hsp70, and calreticulin. *Immunity* 2001 Mar;14(3):303-13.

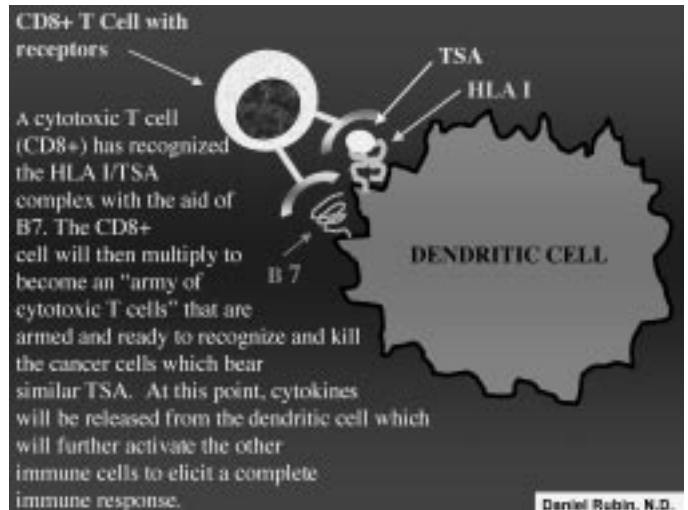


HSP sequence illustration 1: Contact with dendritic cell and entry

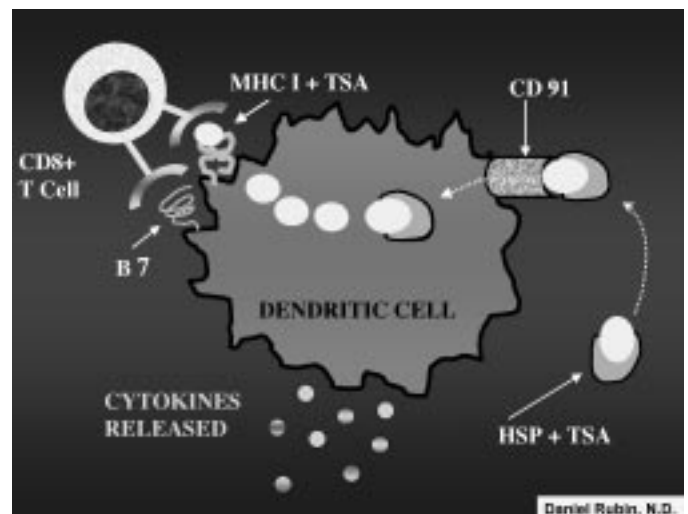


HSP sequence illustration 2: TSA release and transport

phagocytosing cancer cells. In this way, heat shock proteins are crucial for the development of a proper phase I response. Furthermore, HSPs have recently been shown to stimulate the maturation of dendritic cells,¹³ thereby proving their worth towards the phase II response as well. Heat shock protein therapy is easy to perform: heat shock proteins can be elicited from human peripheral blood mononuclear cells, harvested, quantified, admixed with TSA and injected into the skin. The skin is rich in a certain type of dendritic cell called a *Langerhans* cell, which comprise one to three percent of the total epidermal cell population. Due to their location these cells are among the first to encounter foreign antigens including infectious organisms such as viruses and bacteria. Because of this, Langerhans cells are not only easily accessible but are easy to load with antigen. Once the HSP/TSA complexes are injected into the skin, they are usurped by the resident dendritic cells. Once loaded, the immature dendritic cell will migrate deep towards the neighboring lymph nodes, ultimately to present antigen to a large number of cytotoxic and helper T lymphocytes residing in the nodal parenchyma. As mentioned above, the dendritic cells will produce and secrete an array of cytokines that will aid in the progression and potentiation of the local and systemic immune reaction.



HSP sequence illustration 3: Recognition of TSA by cytotoxic T cell



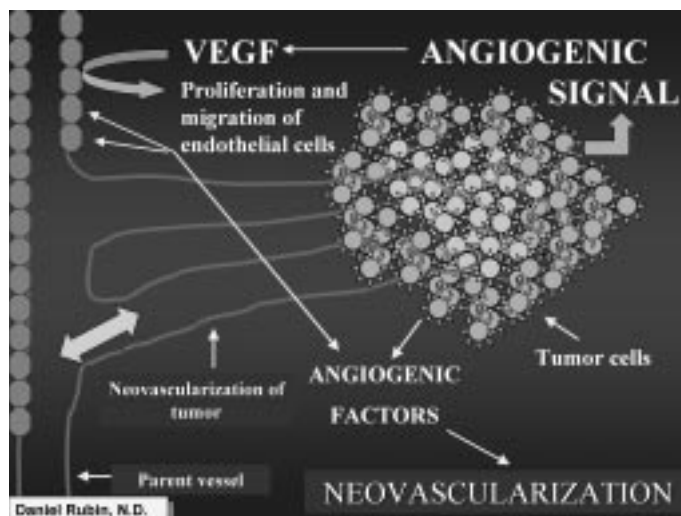
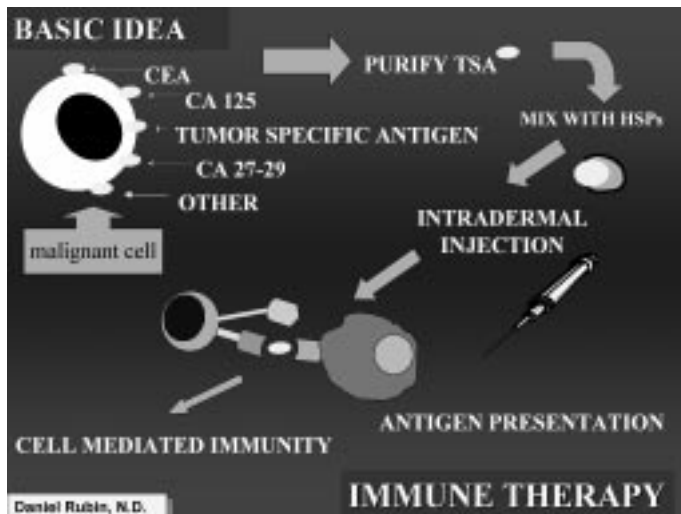
HSP sequence illustration 4: Cytokines released (with overview)

The 'Basic Idea' slide (*top of next page*), gives an overall illustration of the steps involved in the preparation and administration of an immunotherapeutic vaccine. Once TSA is harvested and quantified it is non-covalently associated with HSPs and the combination is injected as a unit. The phase I and phase II cell-mediated cascade follow along ultimately resulting in the development of a cell mediated immune response. The cell mediated immune response is considered the most appropriate endogenous immune response in tumors.

Angiogenesis Inhibition

Angiogenesis inhibition is another way of inducing immunogenicity of tumor cells. Angiogenesis (*growth of new blood vessels*) plays a significant role in cancer¹⁴ since before tumors can grow and metastasize (*spread*) the tumor first needs to be channeled with small blood vessels (*called capillaries*) that allow for delivery of nutrients to the cancer cells and subsequent removal of their metabolic waste. Just as all cells need nutrition to grow, all cells need their waste removed since such waste usually becomes autotoxic. Therefore, growth of new blood vessels in a solid tumor is a survival characteristic of the cancer. Inhibition of the angiogenic

¹³Kuppner MC, Gastpar R, Gelwer S, Nossner E, Ochmann O, Schamer A, Issels RD. The role of heat shock protein (hsp70) in dendritic cell maturation: hsp70 induces the maturation of immature dendritic cells but reduces DC differentiation from monocyte precursors. *Eur J Immunol* 2001 May;31(5):1602-9. ¹⁴<http://www.moffitt.usf.edu/pubs/ccj/v6n5/article2.htm>



process thus provides not only growth control, but also favors regression of the tumor.

Lymphangiogenesis has recently become a recognized phenomenon in cancer progression. Lymphangiogenesis denotes the sprouting of newly vascularized lymph channels and most likely represents the primary mode of cellular waste trafficking from cells. Such channels also serve as routes for metastasis for primary tumor cells, and such for cells harbored in a metastatic deposit. The spread of cancer cells to regional lymph nodes through the lymphatic system is the first step in the dissemination of breast cancer.¹⁵

Previously, understanding of the formation of new lymph vessels has been limited due largely to the lack of lymphovascular-specific markers. More recently, novel, specific markers for lymphatics have been discovered, such as LYVE-1, prox 1 and podoplanin, enabling further research into this new field.¹⁶ Assays for such markers are not yet commercially available. However, one specific marker has been used in humans to detect lymphangiogenesis: vascular endothelial growth factor receptor 3 (VEGFR-3). Two ligands for VEGFR-3, VEGF-C and VEGF-D have been reported to promote tumor lymphangiogenesis and lymphatic

metastasis, and these processes were inhibited by blocking the VEGFR-3-signaling pathway.¹⁷ In several human cancers including those of the breast and prostate, the expression of vascular endothelial growth factor C (VEGF-C) was associated with lymph node metastasis.¹⁸ Furthermore, VEGF has been shown to inhibit immune function. Specifically, VEGF inhibits the maturation of dendritic cells; this mechanism has been touted as one by which cancer cells escape immune surveillance, thus being allowed to grow and prosper.¹⁸ It follows that a decrease in peritumoral or circulating total VEGF will relieve immunosuppression and decrease neovascularization which bears clinical significance reflected by a decreased metastatic potential. Finally, the author has recently reported a decrease in circulating plasma VEGF in a cohort of people with cancer after a mean duration of 16.5 days of combined modality therapy.¹⁹

When angiogenesis is inhibited, cells become deprived of necessary nutrients including oxygen. When this occurs, the cells begin to go through the process of apoptosis,¹⁴ or programmed cell death. When cells become apoptotic, they begin to display certain markers on their cell surface.²⁰ Phosphatidyl serine, normally located on the cytosolic aspect of the phospholipid bilayer membrane and the most immunologically important membrane-bound molecule, becomes displayed on the extracellular surface during the apoptotic event. The phosphatidyl serine then serves as a signal to phagocytic cells to approach and then engulf the apoptotic cell. Promiscuously, as the phagocytic cell engulfs the apoptotic cell, the TSA is engulfed as well. Once inside the phagocytic cell, the TSA becomes recognized and processed the same way it would be if free TSA were taken up by the phagocyte. In this way, angiogenesis inhibition is a form of indirect immunotherapy via augmentation of the membrane characteristics of the cancer cell.

Microfractionated Chemotherapy

As mentioned at the beginning of this article, specific activation of the immune system against an autologous tumor associated antigen is the ultimate route to preventing relapse of a malignancy. During times when a patient has a large tumor volume, this route of treatment is too slow to act. Thus, a more quickly acting metabolic-debulking strategy is necessary. Microfractionated chemotherapy (MCT) represents such a modality wherein conventional cytotoxic agents are utilized at non-cytotoxic doses. Three main purposes are served in this model; when delivered via the microfractionation method conventional agents can be: 1) antiangiogenic; 2) pro-apoptotic; and 3) immuno-stimulatory. Three conventional agents have recently been shown to have angiogenic inhibitory qualities, namely docetaxel and paclitaxel (taxanes) and vinflunine, a vinca alkaloid.²¹⁻²² As above, angiogenesis inhibition will also result in a pro-apoptotic effect on the tumor cells.

MCT provides a general treatment contrast to traditional dosing of the same agents in that the goal of the traditional treatment is to eradicate the tumor cells quickly while the goal of the microfractionation method is to induce a subtle but constant apoptotic effect. In this way, as mentioned above, the tumor cells become more immunogenic. For this reason, MCT is usually *potentiated* with biological response modification (BRMP) with the use of immunothera-

¹⁵Mattila MM, Ruohola JK, Karpanen T, Jackson DG, Alitalo K, Harkonen PL. VEGF-C induced lymphangiogenesis is associated with lymph node metastasis in orthotopic MCF-7 tumors. *Int J Cancer* 2002 Apr 20;98(6):946-51. ¹⁶Cunnick GH, Jiang WG, Gomez KF, Mansel RE. Lymphangiogenesis and breast cancer metastasis. *Histol Histopathol* 2002;17(3):863-70. ¹⁷Kubo H, Cao R, Brakenhielm E, Makinen T, Cao Y, Alitalo K. Blockade of vascular endothelial growth factor receptor-3 signaling inhibits fibroblast growth factor-2-induced lymphangiogenesis in mouse cornea. *Proc Natl Acad Sci U S A* 2002 Jun 25;99(13):8868-73. ¹⁸Ohm JE, Carbone DP. VEGF as a mediator of tumor-associated immunodeficiency. *Immunol Res* 2001;23(2-3):263-72. ¹⁹Daniel Rubin, N.D. Letter to the Editor of *Townsend Letter for Doctors and Patients* July, 2002. Aidan Clinic, Tempe, AZ. ²⁰Dini L, Ruzittu MT, Falasca L. Recognition and phagocytosis of apoptotic cells. *Scanning Microsc* 1996;10(1):239-51; discussion 251-2. ²¹Vacca A, Ribatti D, Iurlaro M, Merchionne F, Nico B, Ria R, Dammacco F. Docetaxel versus Paclitaxel for antiangiogenesis. *J Hematother Stem Cell Res* 2002 Feb;11(1):103-18. ²²Kruczynski A, Hill BT. Vinflunine, the latest Vinca alkaloid in clinical development. A review of its preclinical anticancer properties. *Crit Rev Oncol Hematol* 2001 Nov;40(2):159-73.

peutic and other agents. As the induction of a specific anti-TSA immune response is not immediate, the MCT model enables survival of the patient to the time when such a response can be elicited.²³ By using the microfractionation method the immune systems of patients are left intact thereby able to be stimulated either by immune therapy or by some other mechanism.

Finally, MCT, in itself, acts via an auto-BRMP mechanism. In the past, taxanes have been regarded solely as mitotic inhibitors wherein their antineoplastic activity was characterized by microtubule stabilization. Recently, however, taxanes have been shown to bear antineoplastic activity independent of cell cycle arrest.^{21,24} Paclitaxel, when given at non-cytotoxic dosages, induces a strong increase in circulating tumor necrosis factor (TNF) and a decrease in tumor derived soluble receptors of TNF. The implication here is not only a potentiation of immunologically active cells, but also a release of the inhibition that the presence of soluble TNF receptors places on such cells.

Conclusion

A failure of the endogenous immune response against tumor cells will result in the ultimate demise of its host. Current models of oncological care appear to pay little attention to this since they are mostly focused on rapid destruction of malignant growths with little regard for sequelae to the patient or their immune system. Recent therapeutic advances that support and enable the immune system, however, represent breakthroughs in physiological-based treatments for people with cancer. In time, it is believed²⁵ that these techniques will

obviate current ones as the switch from a treatment-centered/cytotoxic paradigm to a patient-centered/ physiological paradigm occurs. Even with the remarkable advances that have been made in the medical sciences, we still have only glimpses into the wonderful and intricate workings of the human body. Treatments which attempt to attack disease from without, remain, despite their increasing sophistication, blunderings about in a place of marvels. Cancer is a disease that requires individualized treatment that is as physiological as we can attain, with constant reverence for the mystery and beauty of the human body, even in illness.

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ABOUT THE AUTHOR

Dr. Daniel Rubin, N.D., has been with Aidan Clinic since its inception in 1999 as Medical Director and Director of Clinical Research. Aside from treating patients, he plays a significant role in the development of new comprehensive immunotherapies both in the laboratory as well as in the clinic.

Dr. Rubin is the past Medical Director for the Being Alive Wellness Center, the medical program of AIDS Project Arizona as well as a mentor physician for medical students serving two Naturopathic Medical Schools as an Adjunct Clinical Professor. He is a member of the American Association of Naturopathic Physicians (AANP) and frequent lecturer at the AANP Annual Convention.

Dr. Rubin received his B.A. from The University of Iowa and his Doctorate in Naturopathic Medicine from Southwest College of Naturopathic Medicine, in Tempe, AZ, where he also completed his residency. Dr. Rubin frequently lectures in both the academic and professional setting. He has lectured in Asia as well as the United States on Cancer, Immunology and HIV Disease. Dr. Rubin was born and raised in Glencoe, Illinois, a North Shore suburb of Chicago.


²³Charles Knouse, D.O. Personal communication, August 2002. ²⁴Chan OT, Yang LX. The immunological effects of taxanes. *Cancer Immunol Immunother* 2000 Jul;49(4-5):181-5. ²⁵Author's personal opinion.

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