

Temperature Matters! And Why It Should Matter to Tumor Immunologists

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Abstract

A major goal of cancer immunotherapy is to stimulate the generation of long-lasting, tumor antigen-specific immune responses that recognize and destroy tumor cells. This article discusses advances in thermal medicine with the potential to improve cancer immunotherapy. Longstanding evidence indicates that survival benefits are accorded to individuals who achieve an increase in body temperature (i.e., fever) following infection. Furthermore, accumulating evidence indicates that physiologic responses to hyperthermia affect the tumor microenvironment through temperature-sensitive checkpoints that regulate tumor vascular perfusion, lymphocyte trafficking, inflammatory cytokine expression, tumor metabolism, and innate and adaptive immune function. Nevertheless, the influence of thermal stimuli on the immune system, particularly the antitumor immune response, remains incompletely understood. In fact, temperature is still rarely considered as a critical variable in experimental immunology. We suggest that more attention should be directed to the role of temperature in the regulation of the immune response and that thermal therapy should be tested in conjunction with immunotherapy as a multi-functional adjuvant that modulates the dynamics of the tumor microenvironment. *Cancer Immunol Res*; 1(4); 210–6. ©2013 AACR.

*Those who cannot be cured by medicine can be cured by surgery.
Those who cannot be cured by surgery can be cured by heat.
Those who cannot be cured by heat are to be considered
incurable.*

Hippocrates

A Warm-up to the Field of Thermal Medicine

Tumor immunologists often refer to the work of Dr. William Coley over 100 years ago as representing the dawn of modern efforts to stimulate the immune system to combat tumors. Coley witnessed a significant improvement in tumor control and overall survival in patients who were inoculated with bacterial cultures. However, as noted many years later by his daughter, Helen Coley Nauts, and John McLaren in their retrospective review of his work (1), Coley did not appreciate fully that those patients who achieved the longest duration of remission experienced the highest fevers. Moreover, in the mid-1800s, physicians reported (as cited in ref. 2) significant tumor regression in some patients with cancer who experienced high fevers during natural infection by erysipelas, the same bacterial strain that Coley used to inoculate his patients. Unfortunately, Coley's subsequent experiments using more

purified forms of the "toxins," which elicited less fever, failed to elicit tumor regression in contrast to the less purified inoculum. Although fever is a more complex process than simple hyperthermia (or a warming of tissue or core temperature without a regulated change in the hypothalamic set point), an intriguing question remains about the potential contribution of increased body/tumor temperature in antitumor immunotherapy.

The origins of this question are quite old. Long before body temperature could be measured by the first thermometers, clinicians in ancient cultures were aware of the importance of body temperature in health and disease. For example, in ancient Chinese and Ayurvedic medicine, physical or medicinal warming (and cooling) of body temperature was prescribed for such ailments as arthritis and cancer. In ancient Greece, Hippocrates used heat for treatment of a wide variety of inflammatory diseases and cancer and was recognized for prescribing medications derived from the bark and leaves of the willow tree (rich in salicin) to relieve fever; this natural source was used in the 1800s to isolate salicylic acid, the active ingredient in aspirin. Furthermore, the usefulness of external heating to modify vascular function was already known in ancient times. Coley's work not only represents an early effort showing the importance of immune-based therapy but also the modern reinvigoration of efforts to determine the role of temperature in cancer therapies. A growing research community in thermal medicine now focuses on determining the molecular and cellular mechanisms by which temperature manipulation may be used as therapy against cancer and other diseases.

A strong impetus to use hyperthermia in cancer therapy was provided by radiation biologists in the 1970s and 1980s, who showed the potent radiation-sensitization caused by

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hyperthermia; quantifiable cytotoxic effects were induced in tumor cells *in vitro* after heating to heat-shock range temperatures between 42°C and 45°C. Moreover, *in vivo* studies showed that heating increased the oxygenation state of tumors (3). Despite these important findings (3), the broader application of hyperthermia to oncology stalled in the 1990s due to poorly designed clinical trials and the lack of suitable equipment to deliver heat locally. Nevertheless, persistence and gradual improvements in engineering led to several randomized clinical trials evaluating the combination of local/regional heating with radio- and/or chemotherapy. In one trial that combined hyperthermia with radiation, improved local tumor control was achieved in several human or canine cancers (3); in a multicenter randomized trial for patients with localized high-risk soft tissue sarcomas, the regimen combining radio- and chemotherapy with hyperthermia significantly improved clinical outcomes (4).

In the past 20 years, the basic concepts in thermal medicine have greatly expanded. For example, the development of thermosensitive drug-delivery platforms is rapidly transforming the ability to deliver toxic drugs specifically to tumors, avoiding normal tissue (5). The use of isolated limb, intraperitoneal, or thoracic cavity hyperthermia perfusion protocols is increasing (6, 7), whereas image-guided approaches to heating (e.g., magnetic resonance-guided thermal ablation strategies) have the potential to expand even further the therapeutic application of thermal medicine. Radiofrequency and ultrasound-based ablation protocols that create highly defined regions of necrotic tumor tissue through the targeted delivery of cytotoxic doses of heat (or cold, as in cryotherapy) are being combined with other therapies, including immunotherapy (8). Furthermore, interest in the effects of mild (fever-range) hyperthermia on normal and malignant tissues has also intensified, given the impact of this treatment on tumor vascular perfusion, tumor immunogenicity, immune function, lymphocyte trafficking, cytokine activity, metabolism, and gene expression, all of which may affect the tumor microenvironment. Some of these recent discoveries relevant to therapies in the febrile, or mild-to-moderate hyperthermia range (38°C–42°C), are highlighted here. These discoveries suggest that mild hyperthermia may be an effective noninvasive strategy for manipulating the tumor microenvironment in specific ways that could enhance immunotherapy.

Fever and Hyperthermia: Drivers of Thermoregulatory and Immunologic Responses Affecting the Tumor Microenvironment

Thermoregulation is a major homeostatic system in all vertebrates involving extensive neural and vascular networks in the skin and visceral organs to ensure the maintenance of an optimal range of temperatures within the body (9, 10). Mammals and birds are remarkable for maintaining a much warmer core temperature than their environment, which requires substantial metabolic heat production and behavioral modification designed to minimize heat loss from the body surface. Although fever and hyperthermia both involve increases in temperature and changes in thermoregulatory responses, there are major differences in their impact on physiology.

Recognized since ancient times as one of four cardinal signs of inflammation, fever is a complex component of the acute phase response to infection or injury. The increase in body temperature that accompanies fever varies among animals, but involves shifting body functions toward heat production and conservation. For example, the set point for body temperature in the hypothalamus is elevated in most vertebrates during fever, which may result in an animal feeling cold despite an elevated body temperature. Fevers occur in all vertebrates, including those typically considered poikilothermic, such as reptiles and amphibians. Even arthropods and annelids attempt to increase temperature in response to injury and infection. The fact that the "fever" response to infection and injury has been maintained throughout evolution for at least 600 million years strongly suggests a positive benefit to immunity and overall survival (11). The range of temperature elevation through which most vertebrates fight infections is remarkably consistent, varying between 1°C and 5°C above ambient body temperatures (9, 12–14). A central hypothesis of thermal medicine is that there are evolutionarily conserved, highly sensitive, thermal set points that regulate the immune system; These might be manipulated therapeutically to favorably influence the outcome of immunotherapy.

In contrast to fever, hyperthermia results from forced heating of the body or tissues in the absence of a change in the hypothalamic temperature set point, and the body responds by vigorous thermoregulatory cooling mechanisms (9, 10). For example, strenuous exercise and prolonged exposure to warm ambient temperatures can each significantly raise body temperature, initiating thermoregulatory vascular responses. Another major concept being explored by researchers in thermal medicine is that application of heat to a specific region of the body (in the absence of fever) may result in a significant counter reaction aimed at restoring the normal temperature of the affected region. These counter reactions might alter the physiology of the tumor microenvironment, altering the immune response. Both fever and hyperthermia depend upon activation of heat-shock factor 1 (HSF-1; ref. 14), suggesting that a common stress-induced pathway could account for some effects of each process on the immune response.

Here, we provide several examples of research using concepts driven by knowledge of fever and thermoregulation that should be of interest to cancer immunologists. These examples are schematically illustrated in Fig. 1 and highlight mechanisms through which thermal therapy could be strategically used to coopt the natural activities of fever or vascular responses to hyperthermia, to enhance immunotherapy.

Using Heat to Alter Immunosuppressive Hypoxia in the Tumor Microenvironment

There is growing appreciation that the immunosuppressive state of the tumor microenvironment is supported by the hypoxia that develops within tumors (15, 16). Hypoxia is induced by multiple factors within growing tumors, including tumor cell metabolism and abnormal vascular perfusion. When tumor-bearing mice were subjected to temperatures

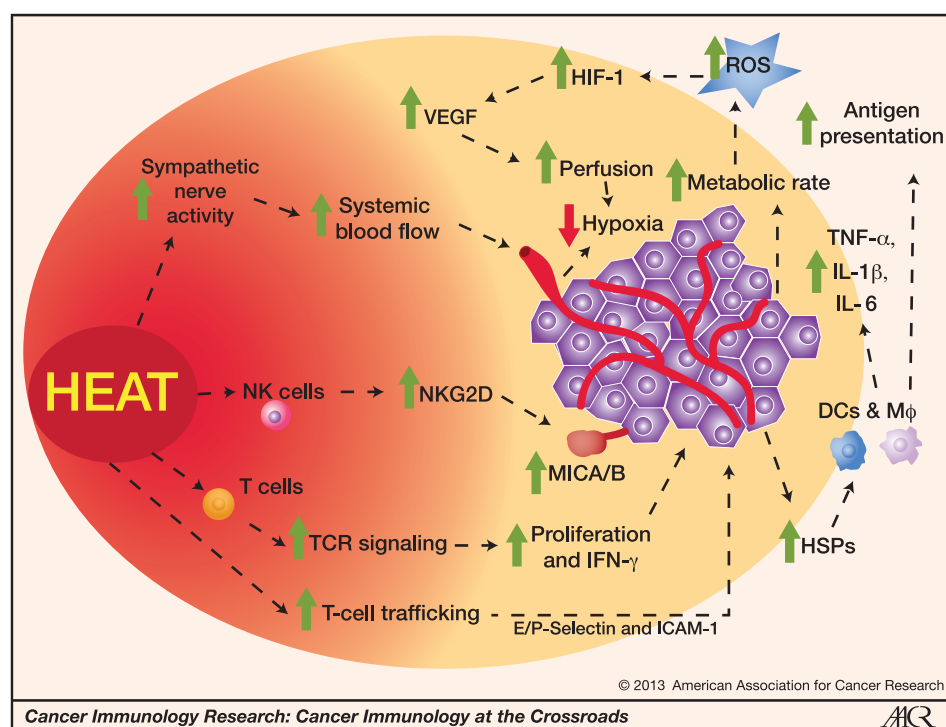


Figure 1. Heating is a multifunctional adjuvant that affects tumor microenvironment through several intrinsic and extrinsic mechanisms, which could enhance immunotherapy in the following ways: increasing vascular perfusion and blood flow to the tumor occurring through both thermoregulatory signals, as well as changes in tumor metabolism, leading to increased hypoxia-inducible factor-1 (HIF-1), which leads to increased reactive oxygen species (ROS) production and VEGF expression; increased trafficking of CD8⁺ T cells occurring through heat-induced increases in E/P selectin and intercellular adhesion molecule 1 (ICAM-1) on tumor blood vessels; increased T-cell receptor (TCR) signaling and differentiation of naïve T cells to effector cells; increased tumor cell-surface expression of MHC class I ligand (MICA/B) and upregulation of the ligand NKG2D on natural killer (NK) cells, increasing NK cell cytotoxic potential; increased functional activity of macrophages and dendritic cells; and increased release of HSPs into the extracellular environment, stimulating downstream immune activity, and increasing antigen presentation. IL, interleukin.

between 39°C and 43°C, increases in tumor oxygenation were observed up to 24 hours after heating (3). The level of reoxygenation correlates with the radiation sensitivity of the tumor as observed in studies with canine sarcomas (17) and in clinical trials of patients with soft tissue sarcomas and breast cancer (18). In the report by Jones and colleagues, one heat treatment led to reoxygenation of tumors in patients within 24 to 48 hours, whereas no measurable reoxygenation of tumors was observed during a week of standard radiotherapy (18). Furthermore, hyperthermia (41°C–41.5°C) induced a significant increase in the pO₂ in hypoxic human tumors, but it did not appreciably affect the oxygenation state of tumors that were not hypoxic (18). In dogs with spontaneous sarcomas, the combination of hyperthermia and radiotherapy led to prolonged improvement in oxygenation in hypoxic tumors (19). These increases in tumor oxygenation improved tumor response to radiotherapy (20). The reoxygenation potential of mild hyperthermia also has important implications for tumor immunology, as hypoxia can support immunosuppressive programs that promote tumor growth and interfere with immunotherapy (16).

Several potential mechanisms by which hyperthermia reduces hypoxia have been identified (3). At the systemic level, Sen and colleagues (21) recognized that addition of heat to large regions of normal tissues surrounding tumors

would activate thermoregulatory responses driven by neural (sympathetic) control of smooth muscle surrounding arterioles, helping to increase blood flow from the heated region to remove excess heat. They hypothesized that blood flow within a tumor would experience at least some of the thermoregulatory "pulse" of blood being actively pumped to the surface during heating. This hypothesis was tested in murine tumor models, which revealed increased perfusion of tumor blood vessels and reduced tumor hypoxia. The interstitial pressure of tumors also decreased following hyperthermia, which may facilitate the infiltration of therapeutic molecules or immune effector cells into tumors. These results provide evidence that targeting a normal vascular response (thermoregulation) using mild, systemic heat treatment can affect the tumor microenvironment and improve the efficacy of radiotherapy.

Hyperthermia can also modify tumor cell metabolism, influencing hypoxia within tumors. For example, hypoxia-inducible factor-1 (HIF-1) has been linked to heat-induced tumor reoxygenation. Moon and colleagues (22) reported that a brief period of hyperthermia activated HIF-1 and its downstream targets, such as VEGF and pyruvate dehydrogenase kinase 1 (PDK1), leading to enhanced tumor perfusion/vascularization and oxygenation. Hyperthermia also increased the transcription of NADPH oxidase-1 through the extracellular signal-

regulated kinase (ERK) pathway, and the augmented expression of HIF-1 and its downstream targets through NADPH oxidase-1-mediated production of reactive oxygen species (ROS). Although much work is needed to optimize the timing of heat treatment to achieve the best outcomes when combined with radio-, chemo-, or immunotherapy, it is clear that targeting tumors with heat can be an effective strategy to change hypoxia and tumor vessel perfusion.

Thermal Therapy Increases Immune Cell Trafficking into the Tumor and Immune Organs

As shown in preclinical mouse tumor models and in human patients, a barrier to CD8⁺ T-cell infiltration in tumor sites frequently exists leading to speculation that tumor blood vessels may be the primary "bottleneck" limiting extravasation of effector cells. Recent studies have shown that mild hyperthermia promotes infiltration of tumor-specific cytotoxic CD8⁺ T cells into the tumor microenvironment (23, 24). Fisher and colleagues examined the rate of cytotoxic CD8 T cell-homing at tumor vascular juncture in several murine tumor models using intravital microscopy (24). They reported that at baseline before hyperthermic treatment, tumor vessels failed to support efficient interactions with circulating T cells. However, these vessels could be converted to high-rate trafficking sites, evidenced by an approximate 5-fold increase in intratumoral homing of cytotoxic T cells following the administration of mild systemic hyperthermia. Notably, mild systemic hyperthermia acts at multiple steps in the adhesion cascade, culminating in improved CD8⁺ effector T-cell trafficking across tumor vascular barriers. Thermally enhanced T-cell trafficking is mediated by E-selectin- and P-selectin-dependent tethering and rolling interactions within vessels walls and stable binding to intercellular adhesion molecule-1 (ICAM-1). A concomitant decrease in the number of intratumoral CD4⁺CD25⁺Foxp3⁺ regulatory T cells (Treg) was observed resulting in an increase in the ratio of CD8⁺ T cells to T reg. These findings show the therapeutic potential of mild systemic hyperthermia treatment (24). Evans and colleagues used intravital microscopy to show that mild hyperthermia also enhanced the capacity of post-capillary high endothelial venules to direct naïve T-cell entry into lymph nodes and Peyer's patches (24–28). Collectively, these observations suggest that thermal signals can exert critical actions in the initiation phase as well as the effector phase of the adaptive antitumor immune response by promoting trafficking within lymph nodes as well as within the tumor microenvironment.

Other Effects of Mild Hyperthermia on Immune Cells

Effects of mild hyperthermia on innate and adaptive immune cells in the tumor microenvironment have been the focus of several recent reviews (29–32). The functions of macrophages, dendritic cells, T cells, B cells, and natural killer (NK) cells may be enhanced after exposure to elevated temperatures. For example, the migration of Langerhans cells to draining lymph nodes is accelerated by mild systemic heating of mice (31); dendritic cells primed with Toll-like receptor (TLR)

agonists increase IL-12p70 production in response to mild heating. Increased temperatures seem to increase TLR4 expression on dendritic cells and macrophages (33). Meinander and colleagues (34) have demonstrated that fever-range hyperthermia significantly influences the persistence of T lymphocytes through regulation of the caspase-8 inhibitor c-FLIP, providing a mechanism by which temperature helps to regulate the dynamics of T-cell repopulation. Treatment of tumor-bearing mice with systemic heating combined with α -galactosylceramide also slowed tumor growth and enhanced survival (35). Hattori and colleagues and Ostberg and colleagues studied mild thermal stress on NK cell cytotoxicity and proposed a mechanism by which thermal stimuli enhance NK cell cytotoxic activity, possibly through increased NKG2D expression along with distinct changes in the overall plasma membrane organization of lipid domains (36, 37).

Cippitelli and colleagues showed that thermally enhanced cytotoxic activity of T cells occurred through modulation of the Fas/Fas ligand system, and was dependent upon thermal activation of HSF1 (38). This effect was also linked to thermally enhanced expression and translocation of the transcription factors AP-1 and NF- κ B, which regulate fas ligand expression. Repasky and colleagues showed that mild systemic heating of mice and *in vitro* treatment of T cells induce the activation of specific protein kinase C isoforms and their movement, along with receptor-activated c kinase 1 (RACK 1) to the T lymphocyte uropod (39).

Although most previous work has not identified specific, antigen-dependent events associated with thermally enhanced immune function, mild thermal treatment can affect subsequent antigen-specific, activation-related events of naïve CD8⁺ T cells. Mace and colleagues observed that exposure of CD62L^{hi} CD44^{lo} Pmel-1 CD8⁺ cells to 39.5°C before their antigen-dependent activation with gp100_{25–33} peptide-pulsed C57BL/6 splenocytes resulted in a greater percentage of cells that differentiated into CD62L^{lo}CD44^{hi} effector cells compared with naïve CD8⁺ T cells incubated at 37°C (40). They also found that mild heating of CD8⁺ T cells resulted in the reversible clustering of GM1⁺ CD⁻ microdomains in the plasma membrane (40, 41). The same membrane clustering phenomenon was also observed in CD8⁺ T cells isolated from spleen, lymph nodes, and peripheral blood following mild whole-body heating of mice (40, 41). In addition, mild heating resulted in the clustering of TCR β and the CD8 coreceptor and enhanced the rate of conjugate formation with antigen-presenting cells (APC) in the spleen. In a pilot study assessing the efficacy of hyperthermic isolated limb perfusion as a treatment regimen for patients with in-transit metastases of malignant melanoma, Olofsson and colleagues found an increase in Melan-A-specific T lymphocytes in a subpopulation of patients, showing the potential of thermal therapy in the activation and differentiation of immune effector cells in the tumor microenvironment (42).

A mechanism by which temperature could regulate the activity of a variety of immune cells could be through thermally sensitive organizational features of the plasma membrane. Because increases in temperature can increase plasma membrane fluidity in a variety of cells, including immune cells, Csoboz and colleagues (43) postulate that increasing the

fluidity of the plasma membrane could be linked to the thermal activation of immune effector cells.

Immunogenicity of Tumor Cells Is Enhanced by Mild Hyperthermia

There is increasing evidence that exposure of tumor cells to hyperthermia enhances sensitivity to immune cell recognition and killing. For example, hyperthermia may enhance the expression of HSPs, now thought to play an important role on the external surface of tumor cells (44). HSP70 family members expressed on the tumor cell surface may activate NK cells via specific receptor interactions. HSP70 stimulates the proliferation and activation of NK cells while binding to Granzyme B, rendering tumor cells more sensitive to NK cell-mediated cytotoxicity.

Additional insight into the molecular mechanisms of thermally enhanced NK cell activity against heated tumor targets comes from studies examining the impact of heat stress on tumor cell expression of the nonclassical MHC class I ligand (MICA), a cell surface protein recognized by an activating receptor (NKG2D) on NK cells (36, 37). The gene for MICA contains heat-shock response elements, which contribute to MICA upregulation upon heat shock. Even mild (fever-range) thermal stress upregulates MICA expression in human colon tumor cells, supporting a role for hyperthermia in increasing the sensitivity of tumor cells to immunologic attack (37).

The role of inducible HSP70 in chaperoning MHC class I epitopes is one mechanism underlying the enhanced sensitivity of tumor cells to immune recognition. HSP70-chaperoned proteins may also stimulate dendritic cells to augment tumor-specific T cells. A prevailing paradigm is that HSP70 released from tumor cells may be in a complex with intracellular polypeptide antigens, which are then recognized by APC, leading to generation of antitumor immunity (45). Although the nature of the HSP70-peptide antigen complex and the APC cell surface receptors involved remain a topic of intense study (46), these interactions may help to regulate a proinflammatory response.

Thermal Regulation of Proinflammatory Cytokines

Findings from several studies suggest that the expression and function of proinflammatory cytokines important for the regulation of antitumor immunity can be modified by mild hyperthermia. Genomic analyses were conducted on tumor biopsies from a cohort of 22 client-owned dogs with spontaneous soft tissue sarcomas treated with thermoradiotherapy (47). Tumor cells were isolated from biopsies obtained before and 24 hours after the first hyperthermia treatment. Chi and colleagues found that changes in the diffusion coefficient of water (a surrogate for inflammation) correlated with differences in several genes associated with inflammation, including CCR1, interleukin-1 β (IL-1 β), IL-6, IL-8, IL-10, and MARCO (47). These results show that clinical application of hyperthermia can lead to an inflammatory response.

Duff and Durum reported that IL-1-induced T-cell proliferation was increased by hyperthermia, suggesting that IL-1

function was sensitive to thermal stimuli (48). However, in addition to being responsive to elevated temperature, this cytokine also functions to increase body temperature in fever. Dinarello and colleagues first identified IL-1 as the endogenous fever producing protein and have postulated that its function could be regulated by febrile temperature (49, 50). Capitano and colleagues (51) recently showed that mild elevation in body temperature (39.5°C) in mice that had been subjected to total body irradiation showed enhanced recovery from neutropenia, involving a thermally enhanced cytokine cascade involving IL-1, IL-17, and granulocyte colony-stimulating factor (G-CSF), which together increased neutrophil production.

Systemic thermal therapy can also alter the activities of the proinflammatory cytokine IL-6 (23, 24) in the tumor microenvironment in a manner that may reduce its contribution to tumor pathogenesis (52). Evans and colleagues discovered that thermal therapy can reverse this protumorigenic function, as IL-6 is involved in the enhanced effector T lymphocyte-trafficking to the tumor microenvironment (23–27). Antibody neutralization of IL-6 but not other inflammatory cytokines present in the tumor microenvironment, such as TNF, IL-1 β , or IFN- γ , prevented the induction of E/P-selectin- and ICAM-1-dependent trafficking of adoptively transferred IFN- γ - and Granzyme B-loaded CD8⁺ T cells via the tumor vasculature. Furthermore, thermal therapy failed to increase ICAM-1 density on tumor vessels or to induce CD8⁺ T-cell intratumoral accumulation in IL-6-deficient mice.

These results not only highlight the potential benefit of adding hyperthermia to various forms of immunotherapy but also to other standard cancer therapies. Indeed, enhanced immune control of tumor growth is revealed when mild heating is combined with chemotherapy (53).

Summary and Future Research Questions

The high grade energy of sunlight becomes the medium grade energy of organic molecules and this in turn is dissipated as the low grade energy of heat, which finally becomes useless as entropy. Thus, the flow of biological energy is irreversible.

Albert L. Lehninger, 1965

Heat is the end product of nearly all of the energy released in the body, and its production is essential for all life. To maintain a very narrow range of core body temperature, the heat produced from metabolic reactions must be constantly balanced with that absorbed from or dissipated to the external environment; this thermal homeostasis is maintained through extensive neural, vascular, and biochemical mechanisms collectively known as thermoregulation. Given the central role of thermoregulation in the physiology of life, it is not surprising that forced addition of heat to the system through hyperthermia or regulated increases in body temperature through fever should have myriad effects on cellular function. Dissecting those effects that may enhance the antitumor immune response and harnessing the power of strategic applications of heat are challenges that researchers in thermal medicine face as they develop tools that can manipulate the temperature of either normal or tumor tissues. One of the

most important aspects of the use of thermal therapy is that it is based on physical interaction with tumor tissue and can therefore avoid the problems associated with adding another potentially toxic drug in various combination strategies. Although this brief overview highlights some of the immune effects of mild to moderate levels of heating, these effects may also be relevant to heating protocols such as thermal ablation, as there are regions of mild hyperthermia at the edges of the ablated field. Several questions must be addressed, however, to maximize the clinical potential of thermal therapies.

One major question is how the thermal energy added to living systems affects overall metabolism and the energy required for antitumor immunity. Recent research shows that there is a large bioenergetic cost associated with generating effective T cell-mediated immune response (54, 55). Results from a new set of studies in mice (Kokolus and colleagues; submitted for publication) show that simply experiencing mild metabolic cold stress even when body temperature remains normal can impair the development of more effective antitumor immunity and significantly increase immunosuppressive activity.

A second question involves the detailed relationships between fever and hyperthermia. The contributions of pyrogen and inflammatory signals from infectious agents to the positive effects of body temperature elevation must still be determined. Once identified, these interactions could potentially be exploited in an adjuvant setting in conjunction with T-cell based cancer immunotherapy or cancer vaccination (23, 24). Moreover, it is clear that we need to identify more precisely how vascular thermoregulatory signals, which can increase tumor perfusion and decrease interstitial fluid pressure (21), can be used to enhance delivery of immune effector cells.

A third question focuses on determining the optimal dose and temperature. Many studies in thermal medicine use temperatures ranging from 38.5°C to 42°C. This is a very large range

physiologically to study the biologic impact of temperature, and much more work is needed to identify the optimal temperatures for influencing various immune endpoints. Studies examining the duration of heating and determining whether local, regional, or systemic heating is most beneficial are also needed.

Finally, development of specific equipment for selective applications of thermal therapy is improving, but much more work is still needed. Whether local, regional, or systemic heating is used will significantly influence equipment design in the future. Furthermore, it is essential that we have rapid, noninvasive techniques for precisely measuring temperatures in various sites in the body (3).

Although these and other questions are important to the applications of thermal medicine, the research highlighted here will hopefully draw attention to temperature as a variable in tumor immunology research. These discoveries have the potential of helping to explain finally, in mechanistic fashion, the natural, long-conserved relationships between thermal modulation and the immune system and, in doing so, they may help to improve immunotherapy in patients with cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Writing, review, and/or revision of the manuscript: E.A. Repasky, S.S. Evans, M.W. Dewhirst

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