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Toward establishment of temperature thresholds for immunological impact of heat exposure in humans

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Abstract

There is interest in understanding the health impact of thermal effects as a result of exposure of humans to radiofrequency/microwave (RF/MW) fields. Immune cells and responses are affected by modest changes in temperature and it is important to quantify these effects and establish safety thresholds similar to what has been done with other tissue targets. Since previous summaries of thresholds for thermal damage to normal tissues have not focused much attention to cells of the immune system, this summary highlights recent studies which demonstrate positive and some negative effects of temperature shifts on human immune cells. We emphasise literature reporting adverse immunological endpoints (such as cell damage, death and altered function) and provide the temperature at which these effects were noted. Whereas there have been many *in vitro* studies of adverse temperature effects on immune cells, there has been limited validation of these temperature effects *in vivo*. However, data from heat stress/stroke patients do provide some information regarding core temperatures (40°C) at which thermal damage to immunological processes can begin to occur. We conclude that there is considerable need for more quantitative time temperature assessments using relevant animal models, more complete kinetic analyses to determine how long immunological effects persist, and for analysis of whether frequency of exposure has impact on immune function. To date, no attempt to categorise effects by using cumulative thermal dose measurements (e.g. cumulative equivalent minutes at a given temperature) has been conducted for cells or tissues of the immune system, representing a major gap in this field.

Keywords

fever; heat stress; hyperthermia; lymphocytes; immune system

Introduction

The goal of this review is to summarise some known effects of mild to moderate temperature increases on cells of the human immune system and to begin to identify potential temperatures and durations at which adverse effects may occur. The human immune system is an interesting target for assessment of how thermal doses affect function

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because cells, tissues and soluble mediators of the immune system, in conjunction with the hypothalamus in the brain, actually participate in the creation of significant natural hyperthermia in the context of fever [1–3]. Fevers can typically result in a 1–4°C increase in core temperature, with the higher temperatures often spiking, or recurring at regular intervals following viral or bacterial infections, often lasting over several days. Unlike tissues such as the brain, which is tolerant only to an extremely narrow temperature range, most of the body, including cells of the immune system, can experience a rise in temperature of several degrees with no apparent long-term damage or thermally induced cell death until reaching 40°C or above. However, even in the case of actual fevers *in vivo*, it is difficult to separate purely thermal effects from those which may result from other immunological mediators, including potent pyrogenic inflammatory cytokines. To learn how the cells of the immune system respond to increased temperatures, several *in vivo* and *in vitro* studies using 'fever-range' hyperthermia have been conducted to assess immunological impact [4, 5]. From these studies it is clear that immune cells remain viable and functional at temperatures of 38.5°or 39.5°C for at least several hours. (Details of these heating protocols are discussed in the sections below.) In addition, there are opportunities to assess effects of hyperthermia on the immune system which may occur during intense exercise or from exposure to RF radiation in the course of 'local/regional hyperthermia' given for treatment of various diseases including cancer [4–6]. Indeed, since important immune cells occupy the tumor microenvironment, further scrutiny of the benefits of local hyperthermia could provide opportunities for answering questions regarding the heat sensitivity and damage thresholds of several different immune mediators. Moreover, with the advent of the use of whole body heating protocols for cancer patients [7, 8], as well as 'deep regional' heating protocols [9], and the recognition that several different immune endpoints may be influenced by warmer temperatures [4, 5], utilisation of these clinical protocols would provide excellent opportunities to validate the effects of thermal exposures determined from *in vitro* studies.

The threshold at which thermal damage may occur to the immune system could also be determined from studies on individuals who suffer from heat stress or heat stroke. If ambient temperatures are very high, heat dissipating thermoregulatory mechanisms may fail after long exposures or after heavy exercise and core temperatures may reach 40°C and above, resulting in severe heat stress and heat stroke. While most studies on thermally induced tissue damage associated with stroke are not focused on particular cells of the immune system, some investigators show that there are altered adhesion properties between endothelium and leukocytes in the blood causing tissue-damaging cellular aggregates within capillaries [10], providing some interesting clinically relevant information on a temperature threshold for the advent of thermal damage to immune cells.

In general, while the number of studies in which temperature has been examined as a variable on human immune cells is increasing, there is a great need to obtain a more quantitative assessment of temperature/duration effects and frequency, and to compare acute versus more long-term effects. To best accomplish this, the immunological studies should be assessed in terms of thermal dose comparisons such as that made possible by the use of cumulative equivalent minutes (CEM) at a given temperature [11] which may include 40°C and above.

The effects of elevated temperature on human immune cell function

Exploring the recent published literature on the effects of heat application on human immune cells reveals several experimental approaches which have been used: assessment of fever and exercise, *in vitro* heating of isolated human cells in a water bath or temperature-controlled incubator, and *in vivo* heating of patients or healthy volunteers with radiant heat sources in the clinic. Fever, for which elevated core body temperature is a hallmark, is a

highly evolutionarily conserved mechanism, suggesting that it provides a survival advantage. Indeed, for some time now it has been well recognised that fever provides a significant survival benefit in several species infected with bacteria [12]. A positive correlation between development of a high fever and survival in humans with cancer was also identified from the analysis of survival data of patients who were treated with fever-causing vaccines in the late 1800 s by William Coley [13]. Because of these and other observations, the thermal component of fever has been studied in mice and humans in order to elucidate the role of elevated temperatures in infections, cancer, and in other diseases [14].

The fact that human immune cell cytotoxicity induced by heating depends on the duration as well as temperature is supported by data collected from monocyte-derived dendritic cells (DCs) obtained from healthy patients. When DCs were heated *in vitro*, the cells maintained viability at 41.5°C for up to 30 min after a 24-h recovery at 37°C [15]. Lethality of these cells was observed at 42°–43°C for treatments of 15 min or longer. It was also noted that extended periods of incubation (1–2 days) at 41°–41.5°C resulted in cell death. To test the protective effect of heat shock on DCs undergoing apoptosis from GM-CSF and IL-4 deprivation, this group observed that following a series of 30 min exposures to 41.5°C, heat-shocked DCs were slightly protected from early apoptosis (Annexin-V+PI-) than deprivation-only controls.

The effect of elevated temperatures on human dendritic cell phenotype and function has also been investigated. Monocyte-derived DCs did not undergo an alteration of immature DC markers (i.e. low or absent HLA-DR, CD80, CD83, CD86) after a series of 30-min heat treatments *in vitro* at 41.5°C with or without lipopolysaccharide (LPS) [15]. Similarly, another group reported that heating of monocyte-derived DCs at 40°C for 7 h did not affect the cell surface expression of MHC I or II, CD40L, CD54, CD80, CD83 or CD86 [16]. Although heat stress at 41.5°C did not affect the antigen uptake ability of immature DCs, LPS-stimulated mature DCs produced significantly more IL-12, p70 and TNF-α during heat shock than controls, migrated further and activated allogeneic naïve T cells with indication of a Th1 response [15].

Selkirk *et al.* [17] and other groups have explored the effect of hyperthermia on heat shock protein expression due to the vital functions of heat shock proteins (HSPs) within eukaryotic cells. HSPs are molecular chaperones that perform and assist with many cellular functions, including folding of nascent proteins, disassembly of damaged or unstable proteins, and mediating antigen presentation [18–20]. In addition, HSPs perform thermotolerance functions during heat stress and can inhibit apoptosis [21, 22]. It is also possible that DCs can be primed by HSPs under certain conditions that may enhance anti-tumour immunity [23]. In studies focused on Hsp70, freshly isolated human peripheral blood mononuclear cells (PBMCs) showed an increase in intracellular Hsp70 at 43°C and extracellular Hsp70 at 40° and 43°C (*in vitro* treatment included a 1 h heat shock followed by 4 h of recovery at 37°C) [24]. Hsp70 release from PBMCs was exosome-mediated, and while there was no difference between the number of exosomes secreted during heat shock, the concentration of Hsp70 was higher than in exosomes from controls.

PBMCs, when exposed to 40°C for 1 h, release about 75% more Hsp70 into the extracellular environment via exosomes than compared to 37°C controls [24]. At 43°C for the same duration there is a 300% increase in extracellular Hsp70. At this time and under these temperature conditions, cell viability was not compromised. Temperature-induced HSP release may contribute to immune modulation through antigen-specific immunity [25].

Similarly to the cytotoxicity studies of DCs, the percentage survival decreases in human polymorphonuclear leukocytes (PMNs) incubated *in vitro* as the temperature was increased from 37° to 39.5°C [26]. At 39°C, PMNs commence apoptosis earlier than cells incubated at 37°C but apoptosis occurs at the same rate in both groups. Apoptosis of PMNs at 39.5°C involves activation of caspases 3, 8 and 9 as well as Bid cleavage.

When heated to 38.5° or 40°C for 18.5 h, macrophages derived from peripheral blood mononuclear cells released significantly less TNF-α following LPS treatment than controls at 37°C [27] with no effect on IL-6. Additionally, macrophages treated at these temperatures for up to 18 h did not disrupt viability, however, [^{3}H]leucine incorporation, at 41°C after LPS stimulation was decreased by about 50%. The human monocytic leukaemia cell line, THP-1, was differentiated with phorbol 12-myristate 13-acetate (PMA) and studied as a macrophage. When pre-treated at 39.5°C for 2 h and stimulated with LPS, increased NF-κB expression as well as TNF-α and IL-6 production was observed. Hyperthermia also increased toll-like receptor (TLR) 4 expression on these cells and it was demonstrated that the enhanced cytokine production was TLR4-dependent, suggesting a mechanism by which mild hyperthermia can activate innate immune responses [28].

Exposure of human mononuclear leukocytes to both 38.5° and 40°C decreased cell viability by 11% and 40% by day 3, respectively, and the effect was further magnified by day 6. However, when the cells were heated for only 2 or 3 h, their bactericidal activity was slightly enhanced [29]. In contrast, Smith et al. [30] found no effect on the viability of human mononuclear cells at 40°C but they observed precocious T cell proliferation and cytotoxicity against target cells when compared with 37°C.

When neutrophils isolated from peripheral human blood are stimulated with LPS and heated on a microscope stage once they had adhered to glass coverslips, the release of reactive oxygen intermediates (ROI) and NO was measured over a period of about 1 min at temperatures ranging from 29°–43°C [31]. Neutrophils heated at temperatures greater than 37°C produced more NO and ROI and this effect was enhanced when neutrophils were stimulated with LPS. This suggests another mechanism by which the innate immune response can be stimulated against invasion of microorganisms.

In studies of other cells of the innate immune response, freshly purified natural killer (NK) cell cytotoxic activity against target cells was enhanced when both cell types were incubated at 39.5°C for 6 h [32]. In these same experiments, the cell surface localisation of NKG2D, an activating receptor on human NK cells, was increased and clustered on the cell membrane, perhaps indicating a mechanism by which thermal stress enhances NK cell function. At shorter exposures to higher temperatures (1 h at 42°C) NK cells isolated from the peripheral blood of healthy donors demonstrated a decrease in cytotoxicity against target cells 5 h following heat shock [33]. Heat shock also selectively decreased perforin expression up to 24 h post-treatment in CD56^{dim} NK cells, but there was no effect on cell viability, or on cell surface expression of CD94, NKG2D, NKp46, CD58 or CD2. Similar repression of NK cell cytotoxicity was reported when NK cells were isolated from cancer patients receiving hyperthermia at 41.5°–42°C for 1 h. However, *in vivo* heating of both healthy patients and those diagnosed with cancer has resulted in an increase in circulating NK cells following hyperthermia at 39.4°–42.2°C [34–37]. These data taken together suggest that hyperthermia can have immunosuppressive or stimulatory effects on NK cells, depending on the treatment protocol, including temperature, duration and timing of heat application. For an extensive review of this subject, see Dayanc et al. [38].

In a study of primary T cell sensitisation to apoptosis by thermal stress, freshly isolated cells that were cultured *in vitro* for 6 days with PHA and IL-2 were susceptible to anti-CD95

following heat shock (40° or 42°C for 2 h) [39]. Hyperthermia treatment did not affect the expression of CD95 or CD95L, however, thermal stress resulted in the degradation of c-FLIP, an inhibitor of apoptosis. Mild hyperthermia and heat shock have been shown to induce FasL gene expression in Jurkat T cells [40]. Both 39.5°C for 4 h and 42°C for 1 h stimulate activated T cells to express FasL, and heat shock induces translocation of NF-κB and AP-1 to the nucleus.

Some of these results have been recapitulated in patients and there are several studies of cancer patients, in particular, who benefit from hyperthermia delivered as an adjuvant to conventional chemotherapy [7, 41, 42]. In a phase I clinical trial of advanced-stage cancer patients who underwent hyperthermia treatment at 39.5°–40°C for 6 h in the Heckel-HT 2000 apparatus, increases in peripheral blood white blood cells (WBCs), CD56+ NK cells and CD69+ activated lymphocytes were observed [8]. In addition to these findings, decreases in circulating eosinophils, lymphocytes, and L-selectin+ cells were reported, and perhaps these changes could be attributed to the activation and recruitment of these cell types to the tumour site. However, during a 1 h heating of seven cancer patients to a core body temperature of 41.8°C, a decrease in circulating CD4+ T cells and an increase in NK cells was observed [37]. Interestingly, following whole body hyperthermia, the decreases in lymphocyte and NK cells correlated with an increase in apoptosis, suggesting that heat has a differential effect on lymphocyte subsets that depends on the timing of the application of the heat.

Exercise-induced heating

Several recent studies of the immune system have evaluated the effects of elevated body temperatures in humans by utilising either exercise or passive heating techniques. Most studies have concentrated on collected data on circulating WBC subsets, with some inclusion of information on cytokine production, if available. Overall, exercise-induced hyperthermia (38°–40°C) seems to correlate with an increase in peripheral WBCs, including lymphocytes, neutrophils and monocytes, and increased pro-inflammatory cytokines [17, 43–46] which suggest an overall benefit on general immune activity from exercise-associated hyperthermia.

Exercise-induced hyperthermia may also provide threshold information about damage caused to the immune system. For example, apoptotic damage of immune cells from heat has also been studied as an endpoint in immune response studies in humans following exercise. Selkirk *et al.* [17] stated that spontaneous apoptosis of CD14+ monocytes increased in untrained subjects between baseline and heat exhaustion (39.1°C, rectal temperature) after exercise. However, CD14+ monocytes isolated from individuals during heat exhaustion and then heat shocked *in vitro* (2 h, 42°C) resulted in decreased apoptosis compared to baseline levels. This also correlated with an induction of Hsp72 expression. Selkirk et al. [17] reported an increase in CD14+ monocyte expression of Hsp72 in trained subjects only as body temperature was elevated. Plasma Hsp72 concentrations increased in untrained and trained subjects from 38° to 40°C. Another group studied Hsp72 protein expression in plasma from participants in a 14-km run who suffered from exertional heat illness (>39°C) [47]. A positive correlation was observed between the increased temperature at the completion of the run and Hsp72 concentration. Additionally, Hsp72 levels were significantly higher in participants who manifested serious indications of heat exertion (e.g. neurological symptoms) than participants who showed mild symptoms.

Other effects of hyperthermia that are detrimental to the immune system are seen after heat stress or in patients with heat stroke and these data provide clear evidence of damaging systemic temperature thresholds. Heat stroke is generally defined as a life-threatening

condition which can be triggered with core body temperature rises to 40°C or higher. The condition occurs with an obvious acute-phase reaction and is accompanied by altered inflammatory and adhesive properties of vascular endothelium, circulating leukocytes and platelets [10]. Evidence shows that heat stroke temperatures are associated with inappropriate endothelial cell activation combined with excessive adhesion and accumulation of leukocytes from the blood, causing tissue-damaging aggregates in the microcirculation. Moreover, data in heat stroke patients shows that the plasma concentration of intercellular adhesion molecule-1 (ICAM-1) and von Willebrand factor are significantly elevated prior to therapeutic cooling, and that following cooling, their levels drop significantly. Other data in patients during heat stress and heat stroke indicate that concentrations of soluble immune mediators, TNF and IL-6 receptors, correlate with hyperthermia and outcome [48]. More recently, confirmation of the role of altered endothelial properties after heat stress/stroke has been obtained in studies using a baboon model [49].

Despite these data it is not clear whether exercise-induced hyperthermia and passive hyperthermia heating techniques designed to mimic physiologically induced hyperthermia result in similar effects on immune cells. For example, several participants in one study were evaluated following passive hyperthermia in a climatic chamber at 45°C and then 50°C to produce rectal temperatures of 38.5°C after 60 min and then 39°C after 1 h [44]. In a second trial, participants exercised for 2 h on a treadmill at 21°–22°C to achieve the same body temperatures as in the first trial. WBCs, neutrophils, lymphocytes, and monocytes were found at statistically increased numbers in the exercise group compared to the passive hyperthermia and controls at 60 and 120 min into the treatment. Additionally, LPS-stimulated whole blood resulted in increases of IL-10 production only in the exercise group. These data suggest that the mechanism by which heat is generated or delivered can result in substantial differences in immunological parameters.

In order to differentiate between the effect of exercise and elevated core body temperature on circulating immune cells, Laing et al. [50] designed a thermal clamp study. During water immersion trials, subjects were heated to 38.4°C over the course of 2 h by exercise in 36.3°C or by passive heating in 38.5°C water. In two control groups, body temperature was maintained at basal levels by exercising in 23.5°C water or passive immersion at 35.3°C. Subjects who exercised in either temperature water had larger increases in circulating neutrophils than those who were passively heated to raise their core temperature. A decrease in bacteria-stimulated elastase release from neutrophils was recorded in both exercise groups as well as the passive hyperthermia group. Taken together, these results suggest that the rise in body temperature alone cannot account for the neutrophil response in the exercise groups.

Although body temperature can be elevated during exercise there are some caveats that are necessary to consider when deriving conclusions about the effect of heat on specific immune cells. The thermal or heat clamp studies [50] are of particular importance in understanding the contribution of the thermal component of exercise on the immune response. Additionally, other variables compound the effects of exercise-induced hyperthermia because catecholamines, cortisol and increase in growth hormone levels during exercise (which may not be increased during passive hyperthermia) and can be associated with increases in circulating lymphocytes [43, 44].

Another important point that should be taken into consideration when studying thermal effects on the components of the human immune system is whether the results in the observed population could be extrapolated to the larger population. Many of the results reported from exercise-induced hyperthermia studies relied on the enrollment of available participants who were mostly small groups of males, and in some cases in exceptional

overall physical health [43, 47]. Perhaps future experimental designs could incorporate the study of a variety of human populations while exploring both quantification of circulating cells as well as the effect of heating on single cell phenotypes, functions, and viability. There is also a need for the study of thermal effects on intact organs of the immune system such as lymph nodes and lymphatic vessels, spleen, and thymus.

While most of the studies identified in this report focus on body temperatures of no more than 38° or 39°C for ethical reasons, it may also be of benefit for future research to study populations of people who are regularly exposed to career-related environmental extremes. This list may include the active military, firefighters, steel workers, and professional athletes such as football players and auto racing drivers who are susceptible to occupational-related heat exhaustion and heat stroke.

Radiofrequency-induced heating

Several studies have focused on elucidating the non-thermal effect of RF energy, highlighting the absorption of lower power at potentially non-thermal levels [51]. The pursuit of non-thermal effects of RF can be attributed, in part, to the observation that many of the adverse biological effects of RF could be ascribed to the accompanying increase in temperature associated with specific absorption rate (SAR) values above 1–2 W/kg in a variety of cell and tissue types [52]. SARs are used to report the deposition of power from a radiofrequency source in tissue. In fact, early studies reported cell cycle-specific sensitivities to cells exposed briefly to 45.5°C using non-RF sources of heat [53]. While cells heated to 45.5°C during S phase demonstrated chromosomal aberrations and cell death rates similar to those of cells exposed to X-ray irradiation; cells at 45.5°C in mitosis or G_1 phase had few chromosomal aberrations yet still died, suggesting that heat damage of proteins, not just DNA could affect cell viability. Additionally, 2 h exposures of human mononuclear leukocytes to either 40.65° ± 0.08°C or 42.73° ± 0.07°C (SARs 12 mW/mL and 22.5 mW/mL, respectively) did not affect cell viability up to 6 days post-treatment, although heating at the higher temperature resulted in decreased DNA, RNA, and protein synthesis 2 to 3 days later in PHA-stimulated cells [54]. In an experiment where heat application for 45 min at 40°C was used as a positive control, Mono Mac 6 (a human monocytic leukaemia cell line) produced increased amounts of superoxide radicals when compared with temperature-controlled, RF-exposed cultures [55].

While a number of studies examined potential adverse effects of RF energy on human immune cells such as cell viability and micronuclei formation, many of these studies have controlled for temperature increases by maintaining culture conditions at 37°C within a few tenths of a degree [56–60]. In some cases when RF-induced heating of immune cells has been allowed or intended, the change in temperature was not reported [61, 62], or unintended temperature variation due to non-uniformity (e.g. the heating of human lymphocytes at 8.8 W/kg) was thought to be difficult to assess and distinguish from the non-thermal component of the experiment [63]. Discussions addressing the most appropriate method to measure sample heating in temperature-controlled environments persist because RF energy is absorbed differently by blood samples than by water, often used as a coolant [59]. Even when attempts are made to maintain temperature, it has also been noted that at SARs above 5.5 W/kg, it is difficult to achieve stable temperature at or near 37°C [64].

However, in the context of setting guidelines for heat exposure for the majority of humans, such variation in temperature may not be applicable. For example, it was reported that mobile communication devices can warm brain tissue about 0.1°C [65, 66], thus prompting the question of whether non-thermal RF can result in changes in neural or ther tissue types. More recently it was suggested that RF exposure guidelines be re-evaluated to provide

temperature-based limitations because subtle changes in physiologic temperature (<0.5°C) can result in molecular changes such as increased HSP expression and membrane lipid organisation [67].

Conclusion

Establishing how RF heating affects cells and functions of the immune system is less well studied than that of other tissue targets, such as the brain. However, the immune system is unique in that this system actively participates in the generation of natural hyperthermia of 1–4°C (i.e. fever) without evidence of damage to immune cells. Indeed, considerable evidence suggests that fever-range temperatures can enhance several immune functions. Thus, it is clear that systemic or regional hyperthermia with an upper limit of 40°C should be well tolerated. However, very little study has been conducted on non-fever-induced hyperthermia, particularly on duration of heating at various mild to moderate fever-range temperatures, or on acute versus long-term immune effects of mild hyperthermia. Establishing time and temperature thresholds for the adverse effects of heat on human immune cells and immune response (such as cell death or altered cell function that contributes to disease pathology, such as inflammation or cancer) is a challenge because of the many variations across studies. The type of heating (e.g. external RF heating, submersion in a water bath, convection or radiant heat), duration of treatment, cell state (e.g. activation with LPS or antigen) and other environmental factors such as whether the treatment was conducted *in vitro* or *in vivo* and whether or not a malignancy was present, can all be confounding factors making direct comparisons difficult.

Given these limitations, it is clear that hyperthermia can be effective at stimulating or enhancing an immune response at mild and moderate fever range temperatures (38–39.5°C) for several hours and can lead to an activation of T cells, NK cells and neutrophils. While heat shock proteins are expressed and released from the cell and may provide protective apoptotic and antigen stimulatory effects, heat shock temperature (41–43°C) durations as short as 15 min can compromise vital cell functions and lead to apoptosis, impairing the ability to synthesise DNA, RNA and protein. While high temperatures (>45°C) have been used locally to ablate tumour cells [68, 69], mild hyperthermia (39–40°C) has been used as an adjuvant to radio- and chemotherapy for a variety of cancers [70–72], and perhaps exudes its positive effects through increased blood vessel perfusion and delivery of therapeutics [73] and by stimulation of immune cells via various mechanisms including heat shock protein and effector molecule expression.

Heat stroke studies in human patients clearly indicate damaging effects of hyperthermia on the vascular endothelium resulting from changes in the expression of adhesion molecules such as ICAM-1, and from these data a clear threshold for damage appears to be at 40°C. Although several studies have examined the effects on cell lines and cell populations isolated from whole blood, more information is needed to discern the effects of heat application on immune cells in their natural environment such as in the spleen, thymus and lymph nodes, for example. It would also be of benefit to measure not only the SAR for RF exposures, but to also correlate these data with the temperature of the exposed tissues.

Clearly, research is needed to help monitor and define relationships between acute and long-term immunological end points, and a well defined ‘thermal dose’ descriptor, which includes a combination of temperature, duration and frequency of exposure, as well as information concerning *in vivo* or *in vitro* applications, is needed. From this data it can be assumed that 40°C should be considered a physiological limit which core temperature should not exceed. Moreover, while a large number of *in vitro* studies have been performed regarding the effects of hyperthermia on various immune cells, fewer experimental studies

have been conducted in humans. However, because of the unfortunate number of patients who experience heat stress or heat stroke each year, valuable data has accumulated which suggests that when internal temperatures reach or exceed 40°C, damaging effects to cells and tissues affecting the immune system (which includes inflammatory and leukocyte functions) can be expected. Needed now are more complete time and temperature analyses *in vitro* and *in vivo* for many different types of immune cells and endpoints. These experiments should also include acute and long-term effects, and determination whether regional or local heating can be tolerated better than heating which increases systemic or core temperatures.

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