Patients with solid tumors treated with high-temperature whole body hyperthermia show a redistribution of naive/memory T-cell subtypes

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Atanackovic, Djordje, Kristina Pollok, Christiane Faltz, Ina Boeters, Roman Jung, Alexander Nierhaus, Klaus-Michael Braumann, Dieter Kurt Hossfeld, and Susanna Hegewisch-Becker. Patients with solid tumors treated with high-temperature whole body hyperthermia show a redistribution of naive/memory T-cell subtypes. Am J Physiol Regul Integr Comp Physiol 290: R585–R594, 2006. First published October 27, 2005; doi:10.1152/ajpregu.00014.2005.—An activation of the immune system might contribute to the therapeutic effect of whole body hyperthermia (WBH) in cancer patients. We explored immune and endocrine responses in patients undergoing high-temperature WBH. Identical parameters were investigated in a separate group of healthy volunteers undergoing physical exercise to rule out effects of sympathetically activated. Lymphocyte subpopulations, lymphocytic expression of a range of adhesion molecules, and serum concentrations of a variety of hormones and cytokines were assessed in cancer patients undergoing high-temperature (60 min at 41.0–41.8°C) WBH (n = 25) and in a separate group of healthy volunteers (n = 10) performing strenuous physical exercise. WBH induced an increase in human growth hormone (hGH), ACTH, and cortisol as well as in TNF-α, IL-6, IL-8, and IL-12R. We observed an increase in natural killer (NK) cells and CD56+ NK T cells shortly after initiation of WBH. In contrast, we found a decrease in T cells expressing L-selectin (CD62L) or αβ integrin adhesion molecules mediating homing to lymphatic tissues. Accordingly, we observed a decrease in CD45RA+CCR7 naive and CD45RA+CCR7 central memory T cells. Numbers of CD45RA+CCR7 memory effector and CD45RA+CCR7 terminally differentiated T cells, on the other hand, remained unchanged. No comparable changes were observed in the group of healthy volunteers. In conclusion, patients with solid tumors treated with WBH show an increase in NK and NK T cells. In a later phase, plasma concentrations of IL-8, hGH, and cortisol increase, correlated with an influx of neutrophils into the peripheral blood. The alterations in T-cell populations suggest that WBH may induce naive and central-memory T cells to enter lymphatic tissue to await antigen exposure and effector T cells to migrate into peripheral tissues to exert their effector function. Although the exercise group may not be an appropriate control to proof the effect of WBH, these changes were not seen in the healthy volunteers performing physical exercise.

immunotherapy; chemokine receptors; fever

WHOLE BODY HYPERTERMIA (WBH) has been used as an adjunct to radiotherapy/chemotherapy in patients with various malignant diseases, and it has been suggested that an activation of the immune system might contribute to the therapeutic effect of WBH. We have previously shown that 41.8°C WBH induces a prolonged T-cell activation in cancer patients (2). In addition, in vitro studies have indicated that the function of certain adhesion molecules expressed by T cells, like L-selectin and αβ integrin, is influenced by treatment with hyperthermia (10, 11, 45). In this study, we investigated whether these and other adhesion molecules play a role for human T-cell responses to high-temperature WBH in vivo.

Previous studies have indicated an increase in different hormonal parameters after WBH, such as ACTH, cortisol, and human growth hormone (hGH) (18, 34). hGH, also known as somatotropin, is mainly produced in the anterior pituitary, but it has been shown to be secreted by lymphocytes and macrophages, as well (46, 48). Plasma concentrations of hGH increase in response to strenuous exercise, but this increase seems to depend on the exercise-related rise in body core temperature (5, 18, 30).

WBH, as well as strenuous exercise, have been shown to be capable of increasing plasma levels of numerous cytokines, such as TNF-α, IL-6, and IL-8 (2, 27, 29, 35). Furthermore, WBH causes an increase in serum levels of sIL-2R, a marker for T-cell activation in humans (2). Both, WBH and exhaustive physical exercise are known to influence the cellular immune system, especially on numbers of peripheral NK and T cells (2, 8, 13, 14, 24, 30, 33). To learn more about the effects of both procedures on the T-cell-mediated immune response, it seemed interesting to analyze whether WBH or physical stress would differently affect naive, central-memory, and effector T cells. Furthermore, an analysis of the expression of adhesion molecules and chemokine receptors would reveal whether WBH and/or physical stress are able to influence the potential of T cells to home into different target tissues. In addition, it seemed to be a challenging task to analyze hormonal parameters, serum cytokines, lymphocyte subpopulations, and T-cell expression of adhesion molecules/chemokine receptors in patients receiving WBH treatment and a group of healthy volunteers undergoing moderate physical exercise, enabling us to answer the question of whether there indeed is a specific effect of elevated core body temperature on different parameters of the immune system.

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In previous studies, we have clearly shown that the immunological effects of WBH are not caused by the intravenous chemotherapy accompanying this treatment modality in our patients with solid tumors (2). However, it has also repeatedly been suggested that the mere physical stress caused by the treatment procedure might be responsible for the immune effects observed in patients undergoing high-temperature WBH (15). To properly address this question, it would have theoretically been required to expose healthy volunteers to both procedures, WBH, and strenuous physical exercise in a cross-over study. Since this approach for ethical reasons seemed not practicable, we alternatively chose to compare our data to a separate group of healthy volunteers performing strenuous physical exercise causing a degree of physical stress comparable to that seen with WBH.

**MATERIALS AND METHODS**

**Patients and healthy volunteers.** A total of 25 consecutive patients undergoing hyperthermia treatment as an adjunct to platinum-based chemotherapy in the Department of Oncology and Hematology at the University Medical Center Hamburg-Eppendorf were included in the study. The majority of patients (16 male, 9 female) suffered from metastatic colorectal carcinoma (n = 12). Other diagnoses included cholangiocellular carcinoma (n = 6), ovarian carcinoma (n = 3), small-cell lung cancer (n = 2), mesothelioma (n = 1), and breast cancer (n = 1). As a separate group, 10 healthy untrained volunteers were recruited from medical staff at the University Hospital Eppendorf in Hamburg. The study protocol was reviewed and approved by the local ethics committee.

**WBH and physical exercise.** Depending on their physical status and the nature of their disease, all cancer patients received 60 min of high-temperature WBH in the range of 41.0 to 41.8°C. All patients received WBH generated by a radiant heat device (Enthermics Medical Systems) in combination with intravenous platinum-containing chemotherapy. One session usually lasted for a total of 4 h (100 min of heating time, a 60-min plateau at target temperature, 60 min of cooling the patient down). In the patients, as well as in the separate group of healthy volunteers, cardiovascular parameters and body core temperatures were monitored continuously. Blood samples were taken before WBH treatment was started, immediately after the 60-min plateau at 41.0–41.8°C, and 0, 3, 5, 24, and 48 h after WBH had been completed.

The physical exercise consisted of continuous treadmill running for at least 140 min. Healthy volunteers were told to perform physical exercise in a degree necessary to reach heart rate frequencies approximately comparable to values derived from historical samples of patients undergoing WBH treatment. Nevertheless, the final analysis of the patients actually included in this study showed that their average heart beat was somewhat below that of the group of healthy volunteers. In the healthy volunteers, blood was drawn before the physical exercise procedure was started and 0, 3, 5, 24, and 48 h after completion of the treadmill running test.

**Flow cytometry.** Absolute leukocyte numbers and differential white blood cell (WBC) counts were determined from 2-ml EDTA blood for all time points using a hematological counter (Beckman Coulter, Krefeld, Germany). Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Biocoll (Biochrom, Berlin, Germany) density gradient centrifugation and washed twice in PBS (Gibco, Paisley, United Kingdom). Cell fluorescence was measured using a FACSCalibur cytometer (Becton Dickinson, Franklin Lakes, NJ). Data analysis was performed using CELLQuest software (Becton Dickinson). Lymphocytes were generally stained with FITC-, peridinin chlorophyll a protein-, or allophycocyanin-conjugated monoclonal antibodies to CD3, CD4, CD8, and CD56 (Becton Dickinson) to determine lymphocyte subpopulations.

To investigate the expression of chemokine receptors and adhesion molecules, we performed costaining with R-phycocerythrin (PE)-conjugated monoclonal antibodies to CCR5, CD62L, α4β7 integrin, cutaneous lymphocyte-associated antigen (CLA), and CCR5 (Becton Dickinson). IgG isotype controls were used in all experiments. A total of 1×10^6 PBMC were washed in PBS, resuspended in 100 μl PBS containing 2% human serum, and stained using the appropriate antibodies according to the manufacturer’s instructions. After incubation on ice for 30 min, cells were washed, resuspended in 500 μl PBS, and were analyzed using a morphological lymphocyte gate.

Costaining for CCR7 expression was performed by first applying 6 μl of a purified mouse anti-human CCR7 IgM antibody. After incubation for 30 min on ice and one wash, 1 μl of a biotin-conjugated anti-mouse IgM (both Ab Pharmingen, San Diego, CA) were added to the cells. After another 30-min incubation period and one wash, 2 μl streptavidin-PE (Immunotech, Marseille, France) were added, and cells were incubated for 25 min on ice.

**Hormonal parameters.** Measurement of cortisol was performed on the Elecsys 2010 analyzer (Hitachi/Roche) using electrochemical detection according to the instruction of the manufacturer [between-run coefficient of variation (CV) was 3.1%]. hGH was determined with the radioimmunoassy hGH-Reaction Indication with Advanced Colour Technology (Schering, Germany) (between-run CV was 5.1%). One nanogram of hGH corresponds to 1.2 μU/1. SI WHO 98/574. The Advantage analyzer (Nichols Institute) was used for measurement of ACTH (between-run CV was 4.3%). For all hormone parameters, quality control samples were used in each of the series.

**Serum cytokines.** Serum was prepared at the given time points from 7 ml of freshly drawn heparinized blood and was stored at −80°C until analysis. Analysis was performed using the Immulite system (DPC Biermann, Bad Neuheim, Germany), a fully automatic random access chemiluminescence-enhanced enzyme immunoassay system. The Immulite assays for sIL-2R, TNF-α, IL-6, and IL-8 are based on a solid-phase, sandwich-enhanced, chemiluminescence-enzyme immunoassay technique. A polystyrene bead, coated with murine monoclonal antibodies specific to the molecule to be measured, serves as the solid phase. Enzyme-labeled polyclonal (rabbit) anti-sera for TNF-α, sIL-2R, and IL-6 are used as detection antibodies. The samples were incubated for 30 (sIL-2R) or 60 min (TNF-α, IL-6, IL-8) at 37°C with intermittent agitation. Unbound components were removed after 30 min using a patented centrifugal washing technique. Automatically added chemiluminescence substrate [3-2-spirodamantane)-4-methoxy-4-(3′-phophoryloxy) phenyl-l,2-dioxetane] was converted by the bound enzyme during the following 10-min incubation period to an unstable intermediate. The resulting light emission was directly proportional to the concentration of the analyte in the samples.

**Statistical analysis.** The significance of observed differences was calculated using the Wilcoxon’s rank sum test. All differences with a P < 0.05 were considered significant.

**RESULTS**

**Body temperature and heart rate.** After initiation of WBH treatment, we observed a continuous increase in the core body temperature in our patients (Fig. 1A). Peak levels of mean body temperature (41.4°C) were reached 140 min after the treatment procedure had been started. In our group of healthy volunteers performing continuous moderate physical exercise, we only observed a minor increase in body temperature measured orally (Fig. 1A). In contrast, the heart rate significantly increased more in our healthy subjects than in our patients undergoing WBH treatment (Fig. 1B), indicating a stronger sympathetic activation caused by the physical exercise. Importantly, this finding indicates that the amount of stress caused by physical
undergoing physical exercise (Fig. 2). Levels of ACTH increased later than hGH levels and reached their peak serum concentration at 3 h post-WBH treatment. In contrast to ACTH levels, which had returned to baseline levels at 5 h post-WBH, cortisol serum concentrations were first elevated at 3 h post-WBH and remained constantly at a high level until the end of the observation period. In contrast, in the control group, levels of ACTH decreased at 5 and 24 h and serum cortisol concentrations were significantly reduced at 5 h postcompletion of one bout of physical exercise (Fig. 2).

Serum cytokines. Already at baseline, serum levels of IL-8 were elevated compared with baseline levels of healthy volunteers. WBH, however, led to a substantial further increase at 0, 3, and 5 h after completion of the treatment procedure (Fig. 3). Serum IL-6 levels increased during the same period of time but, in contrast to IL-8 concentrations, remained significantly elevated until the end of the observation period. Compared with IL-8 and IL-6, serum concentrations of TNF-α showed a later increase at 3 h post-WBH treatment but remained elevated at 5 and 24 h posttreatment. Serum levels of sIL-2R showed the previously described pattern of a transient decrease immediately after WBH treatment followed by a “late” increase at 24 and 48 h post-WBH (2). No significant changes in the serum concentrations of any of the cytokines were observed in the healthy volunteers undergoing physical exercise (Fig. 3).

WBC differential. In a second set of 14 patients undergoing high-temperature WBH, absolute counts of different leukocyte and lymphocyte subpopulations were determined. After a short-lasting decrease immediately after WBH treatment, absolute numbers of WBCs were markedly increased at all time points post-WBH. This phenomenon was caused by a dramatic and prolonged WBH-induced increase in neutrophil numbers and, in part, by a significant increase in monocyte numbers at 3, 5, and 24 h post-WBH. In contrast, lymphocyte counts remained essentially unchanged and were only reduced at 5 h post-WBH. Healthy subjects undergoing the physical stressor showed only minor variations in their WBC differential (Fig. 4).

Lymphocyte subpopulations. Most likely as a consequence of previous chemotherapy treatment for the underlying malignant disease, starting values for most lymphocyte subsets were lower in the patient group. WBH treatment specifically induced a strong and prolonged decrease in the numbers of peripheral

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**Fig. 1.** Body temperature (A) and heart rate (B) in patients undergoing high-temperature whole body hyperthermia (WBH; n = 25) and in healthy controls performing physical exercise (n = 10) before and up to 180 min after treatment had been started. Data are given as means ± SE.

**Fig. 2.** Serum concentrations of different hormones in patients undergoing high-temperature WBH (n = 11) and in healthy controls performing physical exercise (n = 10) before and up to 2 days after treatment was completed. Data are given as means ± SE. hGH, human growth hormone. Asterisks indicate significant (Wilcoxon’s rank sum test) differences compared with baseline value (*P < 0.05, **P < 0.01).
CD4⁺ and CD8⁺ T cells in the patients. T-cell numbers were only back at baseline levels 24 h after WBH treatment had been applied. The numbers of these cells in the group of healthy volunteers, however, remained basically unchanged (Fig. 5). In contrast to the decrease in total numbers of T cells, however, we observed a significant increase in CD4⁺ and CD8⁺ T cells expressing the NK-cell marker CD56 shortly after WBH treatment had begun (Fig. 5). This finding was paralleled by a highly significant increase in NK-cell numbers at the same point in time. In the case of CD56⁺ NK T cells, the increase in peripheral cell numbers was followed by a short-lasting decrease at 5 h post-WBH treatment. Again, none of these findings were observed in the group of healthy volunteers performing physical exercise. Exhaustive physical exercise has been shown to be capable of elevating peripheral numbers of T-helper cells (13, 14, 24, 30, 33). In our healthy subjects performing moderate physical exercise, however, we did not observe any significant changes in the number of CD4⁺ T cells during the course of the experiment. In contrast to this finding, we found a strong and prolonged decrease in peripheral CD4⁺

Fig. 3. Serum concentrations of different cytokines in patients undergoing high-temperature WBH (n = 11) and in healthy controls performing physical exercise (n = 10) before and up to 2 days after treatment was completed. Data are given as means ± SE. Asterisks indicate significant (Wilcoxon’s rank sum test) differences compared with baseline value (*P < 0.05, **P < 0.01).

Fig. 4. White blood cell (WBC) differential in patients undergoing high-temperature WBH (n = 14) and in healthy controls performing physical exercise (n = 10) before and up to 2 days after treatment was completed. Data are given as means ± SE. Asterisks indicate significant (Wilcoxon’s rank sum test) differences compared with baseline value (*P < 0.05, **P < 0.01).
and CD8⁺ T cells in our patients undergoing whole body hyperthermia.

Adhesion molecules and chemokine receptors. While naive and “central memory” T cells usually circulate between the peripheral blood and lymphoid tissue, effector T cells home to peripheral tissues to await reexposure to pathogens (21). Analysis of T-cell subpopulations expressing different adhesion molecules and chemokine receptors revealed that WBH treatment induced a decrease in peripheral numbers of CD4⁺ and CD8⁺ T cells expressing L-selectin (CD62L), a homing receptor for lymphoid tissue (Fig. 6). In our subjects performing physical exercise only, a short-lasting decrease in CD4⁺ and CD8⁺ T cells was observed immediately after that exercise procedure had been completed.

In the cancer patients, a decrease comparable to the one seen for CD62L⁺ T cells was observed for CD4⁺ and CD8⁺ T cells expressing α4β7 (Fig. 6), another molecule initiating the migration of blood-borne lymphocytes into lymphoid tissues (7). Again, in our subjects performing physical exercise, only a short-lasting decrease in CD8⁺ T cells was observed immediately after the exercise procedure had been completed. We also observed a significant drop in CD4⁺, and to a lesser extent, CD8⁺ T cells expressing the skin-homing receptor CLA (Fig. 6). No comparable changes were observed in the healthy subjects undergoing physical exercise.

In contrast to the above mentioned findings, peripheral numbers of T cells expressing CCR5 decreased to a much lesser extent and only for a short period of time in our patients undergoing WBH (Fig. 6). Interestingly, this chemokine receptor, in contrast to CD62L and α4β7 integrin is preferably expressed by antigen-experienced effector-type T cells (12, 38, 42).

It has recently been suggested that the pattern of expression of the lymph node homing receptor CCR7 and CD45RA divides human CD4⁺ and CD8⁺ T cells into distinct subsets (37). Both, the CD45RA⁻CCR7ⁿaïve, as well as the CD45RA⁻CCR7central memory fractions, circulate between the peripheral blood and lymphoid tissue and have no significant effector potential. In contrast, CD45RA⁻CCR7effector memory and CD45RA⁺CCR7 terminally differentiated effector cells do not enter lymphatic tissue but are equipped with an arsenal of effector mechanisms to be able to exert their function in peripheral tissues. We observed a drastic and prolonged decrease in naive CD45RA⁻CCR7 and CD45RA⁻CCR7 central memory T cells in patients undergoing WBH treatment (Fig. 7). As explained, both of these CCR7⁺ subpopulations are known for their potential to home to secondary lymphoid tissues. In contrast, numbers of CD45RA⁻CCR7 memory effector CD4⁺ T cells remained unchanged over the course of the treatment. Memory effector CD8⁺ T cells only showed a comparably late decrease only at 5 h post-WBH. Remarkably, the peripheral numbers of CD45RA⁺CCR7⁻ terminally differentiated CD4⁺ and CD8⁺ effector T cells remained stable over the whole observation period. No comparable pattern of redistribution of
peripheral lymphocytes was observed in the group of healthy volunteers.

**DISCUSSION**

Our previous studies have clearly shown that the immunological effects of WBH are not caused by the intravenous chemotherapy accompanying this treatment modality in our patients with solid tumors (2). However, it has also repeatedly been suggested that the mere physical stress caused by the treatment procedure might be responsible for the immune effects observed in patients undergoing high-temperature WBH (15). Whereas differences in the baseline levels of ACTH, cortisol, IL-8, sIL-2R, and T cells indicate differences in the general immune status between both groups, treatment-related effects on hormonal and immune parameters were still only seen in subjects receiving WBH. Given the fact that the amount of physical stress, as judged by the increase in heart rate on the other hand, was even lower in WBH-treated patients, we therefore conclude that the immune effects of WBH seem to be related to the increase in body core temperature and are not caused by pure stress as defined by an increase in sympathetic activation.

Vigorous physical exercise through an increased metabolic heat production typically induces a 1–4°C increase in body core temperature (5). In our untrained healthy subjects, however, the relatively mild exercise did not cause major changes in body temperature. This might explain why we did not observe any significant changes in hormonal or immune pa-
parameters that have been described by other groups applying a much more exhausting form of physical exercise. Nevertheless, the results have to be interpreted with caution, because, for ethical reasons, it was not possible to also compare them directly to a group of healthy volunteers undergoing WBH. Only WBH treatment, but not strenuous physical exercise, caused a significant increase in different hormonal parameters. Triggered by the WBH treatment, a release of ACTH was induced in our patients. ACTH, in turn, seemed to have induced an increased production of cortisol by the adrenal cortex, a finding in line with previous finding by Robins et al. (34). Interestingly, cortisol is known to stimulate the migration of neutrophils from the bone marrow into the circulation (5).

As pointed out earlier, exercise-related increases in plasma concentrations of hGH seem to be related to a rise in body temperature. Therefore, we suggest that the small increase in body temperature in our healthy volunteers did not suffice to generate a significant increase in plasma hGH. In our patients undergoing WBH, however, the increase in body core temperature led to a significant rise in plasma hGH levels, which in turn might have contributed to the peripheral neutrophilia observed in these patients. This idea is supported by the finding
that an intravenous infusion of growth hormone induces a marked neutrophilia (5, 28) and by the fact that a heat-induced increase in peripheral neutrophil numbers can partly be abolished by blockade of hGH release by somatostatin (19).

In this study, we confirm previous findings by us and others demonstrating an enhancing effect of WBH treatment on the release of different cytokines in humans (2, 35). As in our previous study (2), we observed a prolonged increase in serum levels of TNF-\(\alpha\) in patients undergoing high-temperature WBH treatment. It has been indicated that in vivo hyperthermia augments monocyte production of TNF-\(\alpha\) (49). However, we suggest, that T cells activated by the WBH-treatment might have also contributed to the increase in serum TNF-\(\alpha\) levels in our patients. This idea is based on the results of our previous study showing that WBH causes an activation of peripheral T cells in humans and also induces an increase in serum levels of sIL-2R, a marker for T-cell activation in humans (36), a finding which is confirmed by observations described in the present study.

Activated T cells are capable of augmenting the production of IL-8 by monocytes, a major source of this cytokine (43). In addition, IL-8 is produced by a variety of other cells including neutrophils, mast cells, endothelial cells, and different tumor cell types. IL-8 is a proinflammatory cytokine, whose principal role in infection and inflammation appears to be the recruitment and activation of circulating and tissue neutrophils and lymphocytes to the site of tissue damage (40). IL-8 has been shown to play important roles in human tumor progression, and increased levels of IL-8 have been found in the serum of patients with different cancers (47). In our patients with solid tumors, we also observed increased baseline serum concentrations of IL-8 compared with healthy volunteers and the levels of IL-8 in the cancer patients further increased after treatment with WBH. It remains unclear whether this increase was the consequence of a WBH-induced increased production of this cytokine by cells of the immune system, tumor cells, or both. It seems likely, however, that the elevated production of IL-8 contributed to the neutrophilia seen in our patients, a phenomenon that has also been observed after intravenous application of this cytokine where this effect is caused by a rapid mobilization of neutrophils from the bone marrow into the peripheral blood (44).

IL-6, which is also induced by WBH treatment, exerts a wide spectrum of biological actions, including regulation of immune responses, hematopoiesis, and acute-phase reaction. In addition, IL-6 has marked stimulatory effects on the hypothalamic-pituitary-adrenocortical axis resulting in increases in ACTH and cortisol secretion (23). Therefore, it seems possible that the WBH-induced increase in serum IL-6 was partly responsible for the increase in ACTH and the subsequently enhanced secretion of cortisol in our patients.

Strenuous exercise is generally capable of increasing plasma levels of cytokines-like TNF-\(\alpha\), IL-6, and IL-8 (27, 29). However, in our study, we did not observe any significant changes in serum levels of these cytokines in the group of healthy volunteers performing physical exercise. One reason for this might be that the intensity of the exercise applied in our study was less pronounced than in other studies. Our findings indicate that the body core temperature might be a major mediator of immunological responses. Accordingly, it has previously been shown that a combination of exercise and that heat stress augments both hormonal and immune responses. These changes, however, seemed to be reversed if temperatures are clamped by exercising in cold water (5, 30, 32, 33), suggesting that an exercise-induced threshold of \(\sim 38^\circ\text{C}\) body core temperature exists where hormonal and immunological consequences of exercise can be observed (32).

As has been described earlier, WBH treatment has major effects on the cellular immune system (8). In this study, we repeated our earlier observations demonstrating a prolonged WBH-induced increase in peripheral leukocytes caused by a significant increase in neutrophils in the peripheral blood (2). As in the previous study, this increase lasted at least 2 days. It is a well-known fact that physical exercise is also capable of inducing an increase in circulating neutrophil count (5). This observation is reflected by the increase in total WBC and neutrophil numbers in our healthy subjects. However, in contrast to our patients undergoing WBH, levels of peripheral neutrophils quickly returned to baseline levels and even dropped to a concentration below baseline at 24 h postexercise.

As demonstrated in the present as well as in previous studies, high-temperature WBH has the potential to induce a significant but short-lasting rise in the number of peripheral NK cells during and shortly after WBH treatment (2, 9). In contrast to T cells, NK cells do not seem to recirculate between the peripheral blood and peripheral tissues. However, trafficking of NK cells from the circulation to the tissues has been demonstrated after the infusion of cortisol and IL-6. Therefore, it might be that the hyperthermia-induced increased serum levels in these agents might also have the potential to induce this kind of NK cell relocation. This hypothesis might also be supported by observations in mouse models where increased numbers of NK cells were found at the tumor site after hyperthermia treatment (6).

In this study, we observed marked WBH-induced increases in both CD4\(^+\) and CD8\(^+\) T cells expressing the NK cell marker CD56. This finding might be of clinical relevance, since it has been proposed that CD8\(^+\) T cells expressing CD56 represent the currently circulating effector lymphocytes (31) specific, i.e., for viral (17, 41) or tumor antigens (41). In addition, CD56 has been shown to be expressed by peripheral (26, 39) and tissue-infiltrating (3) CD4\(^+\) T cells with a Th1 cytokine profile and a strong cytotoxic potential. This finding is in agreement with our previous observation of high concentrations of intracellular granzyme A within these CD4\(^+\) T cells (1), a protein that is found in the cytoplasmic granules of cytolytic cells (4).

WBH has repeatedly been shown to induce a decrease in the number of CD4\(^+\) (9) and CD8\(^+\) T cells (2, 9) in the peripheral blood. It has been examined whether an increased apoptosis rate of T cells might contribute to this phenomenon. The percentage of T cells undergoing apoptosis after WBH treatment, however, is so small (9) that this observation cannot explain the decrease in the peripheral number of these cells. We suggest that a different mechanism is responsible for the WBH-induced decrease in T cells within the peripheral blood.

Adhesion molecules and chemokine receptors can be up-regulated or lost as cells differentiate, allowing leukocytes to coordinate their migratory routes with their immunological differentiation state. Thus tissue-homing effector T cells express inflammatory chemokine receptors like CCR5. In contrast, naive and central memory T cells, that in searching for
antigen circulate between secondary lymphoid organs and the peripheral blood, express homing receptors for lymphoid tissue like CCR7 or CD62L (16, 22). Another molecule initiating the migration of blood-borne lymphocytes into lymphoid tissues is α4β7 integrin, which specifically plays a role in tissue-specific homing of T cells to Peyer’s patches but also to the intestinal lamina propria (7).

Recent studies have shown that exposure of animals to intermediate-temperature hyperthermia profoundly increases 1-selectin and α4β7 integrin-dependent trafficking of lymphocytes to secondary lymphoid tissues (11), resulting in increased numbers of T cells within lymph nodes several hours post-WBH treatment (25). In line with a recent study by Kraybill et al. (20), we observed a significant and prolonged WBH-induced decrease in the peripheral numbers of both CD4+ and CD8+ T cells expressing CD62L. At the same time, T cells expressing α4β7 integrin decreased in our patients undergoing WBH treatment. In contrast, peripheral numbers of T cells expressing CCR5 decreased to a much lesser extent and only for a short period of time. CCR5, in contrast to CD62L and α4β7 integrin, is preferably expressed by antigen-experienced effector-type T cells (12, 38, 42).

Based on these findings and the above-mentioned in vitro studies, we suggest that WBH treatment might specifically lead to a redistribution of different naive and memory-type T cells and that this mechanism may be responsible for the observed reduction in total numbers of peripheral T cells after WBH. In this scenario, WBH would cause a specific relocation of less differentiated naive T cells into lymphatic tissue to encounter antigen, while antigen-experienced effector-type T cells would remain in the peripheral blood, in case of local inflammation, to home to different peripheral tissues. To further test this hypothesis, we determined the numbers of different memory/effector T-cell subtypes, defined by their expression of CD45RA and CCR7.

We observed a strong and prolonged decrease in naive CD45RA+CCR7+ and CD45RA−CCR7− central memory T cells in patients undergoing WBH treatment. In contrast, numbers of CD45RA−CCR7− memory effector CD4+ T cells remained unchanged over the course of the treatment. Memory effector CD8+ T cells showed a comparably late decrease only at 5 h post-WBH. Importantly, the peripheral numbers of CD45RA+CCR7− terminally differentiated CD4+ and CD8+ effector T cells remained unchanged over the whole observation period.

In conclusion, our present study provides evidence for an additional mechanism through which hyperthermia might exert its effects on the host’s immune system. In addition to its multiple effects on the human cytokine network, hyperthermia might lead to redistribution of immune effector cells within the human body.

We hypothesize that an immediate change induced by WBH is an increase in the circulating counts for NK cells and NK T cells. In a later phase after WBH treatment, plasma concentrations of IL-8, hGH, and cortisol also increase. This causes an influx of neutrophils from the bone marrow. At the same time, the heat exposure induces naive and central memory T cells to enter lymphatic tissue to await antigen expression and effector T cells to leave the circulation and migrate into peripheral tissue where they exert their effector function. Future studies in the preclinical setting should address the potential of WBH as an attractive adjuvant for patients undergoing active immunotherapy, e.g., treatment with cancer vaccines.

GRANTS

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