

Can Exercise-Induced Modulation of the Tumor Physiologic Microenvironment Improve Antitumor Immunity?

Xiaojie Zhang¹, Kathleen A. Ashcraft¹, Allison Betof Warner², Smita K. Nair¹, and Mark W. Dewhirst¹



Abstract

The immune system plays an important role in controlling cancer growth. However, cancers evolve to evade immune detection. Immune tolerance and active immune suppression results in unchecked cancer growth and progression. A major contributor to immune tolerance is the tumor physiologic microenvironment, which includes hypoxia, hypoglycosis, lactosis, and reduced pH. Preclinical and human studies suggest that exercise elicits mobilization of leukocytes into circulation (also known as "exercise-induced leukocytosis"), especially cytotoxic T cells and natural killer

cells. However, the tumor physiologic microenvironment presents a significant barrier for these cells to enter the tumor and, once there, properly function. We hypothesize that the effect of exercise on the immune system's ability to control cancer growth is linked to how exercise affects the tumor physiologic microenvironment. Normalization of the microenvironment by exercise may promote more efficient innate and adaptive immunity within the tumor. This review summarizes the current literature supporting this hypothesis.

Introduction

Although widely used to manage chronic diseases (1, 2), incorporating exercise into cancer therapy is relatively new. Epidemiologic studies have shown that aerobic exercise reduces cancer incidence and progression after diagnosis, in a variety of malignancies (3–6). Clinically recommended exercise levels (e.g., 150 minutes of moderate exercise per week) are associated with up to 40% risk reduction for developing breast and colon cancers (4, 7, 8). Part of these effects may be related to how exercise affects antitumor immune function (9–11).

Preclinical studies have revealed how exercise changes the physiologic tumor microenvironment. For example, exercise reduces tumor hypoxia and improves vascular maturity and perfusion (12–15). It is well established that tumor hypoxia contributes to tumor progression, radioresistance, and chemoresistance. Improved perfusion and reduced hypoxia in the tumor microenvironment could improve drug delivery, enhance tumor response to chemotherapy, and lead to better prognosis (12, 16–18).

Current FDA-approved immune-based therapies for cancer are, in large part, designed to reverse tumor immune escape and tumor-induced immune suppression (for example, immune checkpoint blockade; ref. 19). Despite significant success with

immune-based therapies, a substantial proportion of patients do not respond (20). Thus, there is a rationale for adjunct strategies to improve clinical benefit associated with immunotherapy (21, 22).

Figure 1 summarizes our hypothesis that exercise-induced normalization of tumor microvasculature, hypoxia, and metabolism promotes competent cytotoxic immune cell infiltration into tumors.

Modulation of the Tumor Microenvironment by Exercise

Immune cell trafficking into tumors

Aberrant tumor vasculature downregulates endothelial adhesion molecules, which impairs leukocyte entry into tumors (23, 24). The normalization of tumor vasculature increases endothelial cell adhesion molecule expression, facilitating leukocyte entry into tumor parenchyma (25, 26). In murine tumor models, the combination of anti-PD-L1 and vascular endothelial growth factor (VEGF) blockade increased the density of high endothelial venules and caused a 3- to 10-fold increase in tumor-infiltrating T and B lymphocytes (26). Vascular normalization of tumors also occurs following exercise in murine models of cancer (12, 16). Therefore, exercise-induced normalization of tumor vasculature may increase endothelial adhesion molecule expression and promote immune cell infiltration into tumors.

The effect of exercise on immunity toward infection

Many recent reviews have highlighted the role of exercise in maintaining a healthy immune system and controlling infections and chronic inflammation-associated disease (11), including cancer (9, 10, 27, 28). Moderate exercise in mice (20–30

¹Duke University Medical Center, Durham, North Carolina. ²Memorial Sloan Kettering Cancer Center, New York, New York.

S.K. Nair and M.W. Dewhirst share senior authorship of this article.

Corresponding Author: Mark W. Dewhirst, Duke University Medical Center, Box 3455 DUMC, Medical Sciences Research Building, Room 201, Durham, NC 27710. Phone: 919-684-4180; Fax: 919-684-8718; E-mail: mark.dewhirst@duke.edu

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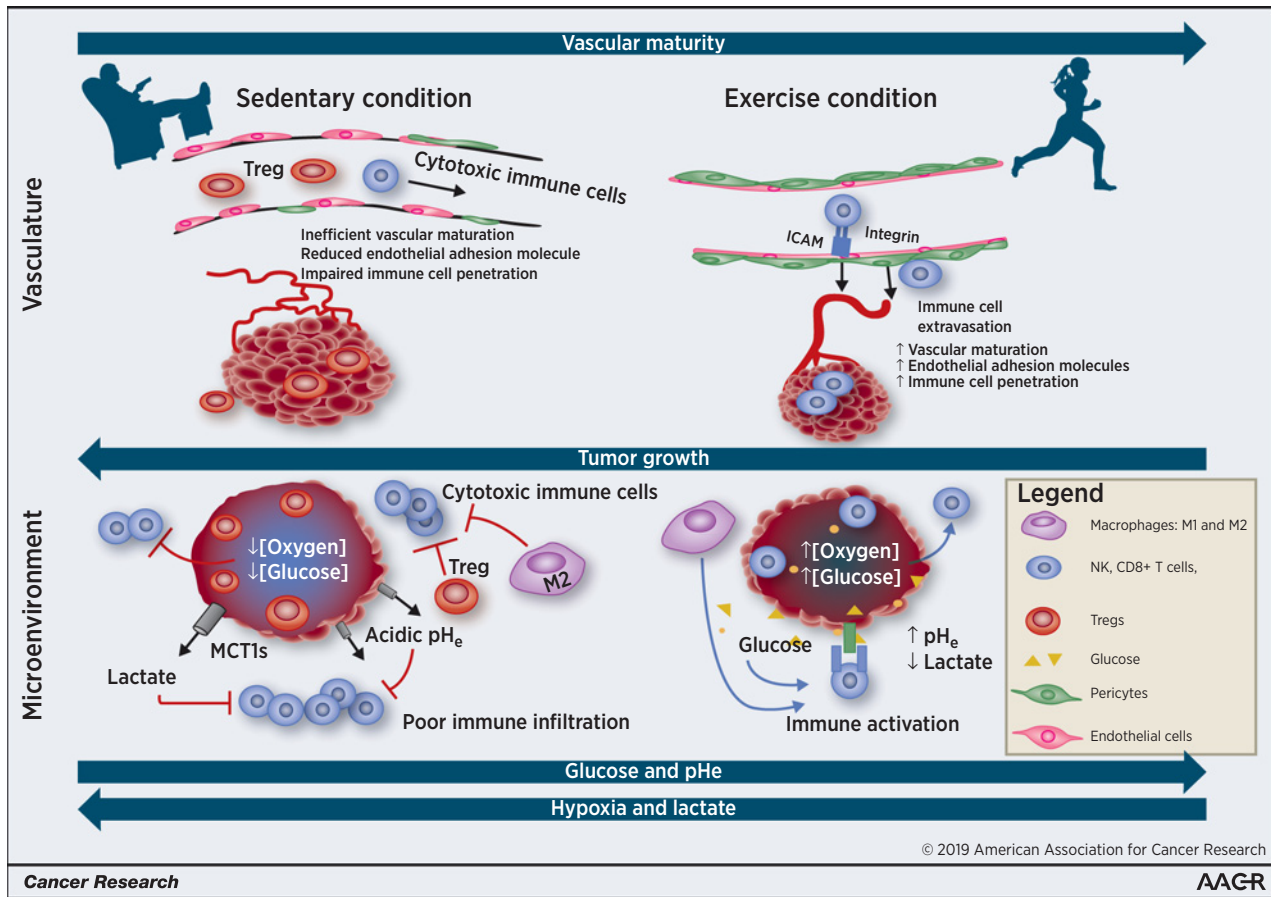


Figure 1.

Exercise primes the tumor toward a more aerobic, less glycolytic physiologic microenvironment. The schematic demonstrates the large-scale vascular (top) and microscale physiologic microenvironment (bottom) changes within the tumor of an exercised versus sedentary individual. It is not drawn to scale. Exercise, in normalizing tumor vasculature, increases endothelial adhesion molecule expression, promotes the extravasation of cytotoxic immune cell (NK cells, CD8⁺ T cells, and type 1 macrophages) and infiltration of these cells into the tumor. Conversely, under the sedentary condition, tumors manifest a hypoxic, aberrantly vascularized, and highly glycolytic tumor. The two tumor cross-sections on the bottom represent the physiologic microenvironment of a sedentary versus exercised individual. High lactate, low pH_e, and hypoglycotic environment within the sedentary tumor promote immune-suppressive Tregs but inhibit the function of tumor-infiltrating cytotoxic immune cells. The immune cells that are in the sedentary tumor microenvironment are metabolically outcompeted by the highly proliferative cancer cells. Exercise, by normalizing tumor vasculature, yields a better perfused tumor, with improved energy substrate (glucose availability), improved oxygen concentrations, and decreased glycolytic lactate production. The net effect of this is an enhanced metabolic and immunologic environment, one that results in more potent immune activation and more effective tumor cell cytotoxicity.

minutes/day of treadmill running) increased the survival rate by 2-fold following influenza infection compared with inactivity. In contrast, intense exercise (2.5 hours/day of treadmill running) increased morbidity (29). Epidemiologic human studies support moderate exercise as more beneficial to immune function than intense exercise (30). Women who walked briskly for 45 minutes 5 days/week had reduced duration of upper respiratory tract infection (URTI) symptoms compared with sedentary counterparts (5.1 days vs. 10.8; ref. 31). However, marathon runners who averaged >96 km/week had 2-fold higher odds of URTI compared with runners who averaged 32 km/week (32). Results such as those led to an "open-window" hypothesis: following vigorous exercise, an individual is transiently immunosuppressed (33). A recent review by Campbell and Turner argues that acute/vigorous exercise is not immunosuppressive; in the long run, exercise improves immune cell function, and exercise-induced immune

cell redistribution is beneficial (27). This hypothesis is coined the "acute stress" or "exercise immune-enhancement" (27, 34).

Studies that examined the effect of exercise on immune cell phenotype and function in preclinical and clinical settings are listed in Tables 1A and 1B.

Exercise and the innate immune system

Exercise studies reported increased natural killer (NK) cell and macrophage reactivity against tumors. NK cells directly kill tumor cells via a perforin-dependent mechanism (35, 36). Subsets of NK cells are also involved in cross-talk with dendritic cells (37). This cross-talk can enhance antigen presentation and downstream effector cell responses (38–41). Pedersen and colleagues demonstrated that voluntary wheel running halved tumor incidence compared with sedentary controls in mice with diethylnitrosamine-induced liver tumors (42). Further, exercise prior to

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Table 1A. Preclinical publications reporting immunologic effects in tumor-bearing mice associated with exercise

Article	Model	Exercise modality	Tumor growth effects of exercise	Immune effects of exercise
Innate immune response adaptation to mice subjected to administration of DMBA and physical activity Abdallah et al. 2014 PMID: 24520305	BALB/c with DMBA induced mammary tumor	Swim training 5 days/week for 8 weeks in both no tumor and mammary tumor mice	Not addressed	↓IL12-producing macrophages ↑IFN γ ↑M1 cytokines ↑TNF- α ↓TGF- β
Chronic exercise increases macrophage-mediated tumor cytotoxicity in young and old mice Lu et al. 1999 PMID: 9950928	Young (6 mo) and aged (22 mo) BALB/cByJ mice, nontumor bearing	Treadmill running sessions for 16 weeks	Not addressed	↑Macrophage cytolytic activity ↑NO $^-$ production by macrophages among young mice with exercise
Exercise training in transgenic mice is associated with attenuation of early breast cancer growth in a dose-dependent manner Goh et al. 2013 PMID: 24312199	PyMT transgenic mouse with mammary carcinoma	Voluntary wheel running starting at 42 days of age, until tumor becomes fulminant	↓Tumor volume with exercise in first 4 weeks by 50%	↓CCL22
Voluntary running suppresses tumor growth through epinephrine and IL6-dependent NK cell mobilization and redistribution Pedersen et al. 2016 PMID: 26895752	C57BL/6 mice with B16F10 tumors and Lewis Lung carcinoma mice	Voluntary wheel running before, after, or during tumor challenge	↓B16F10 tumor growth by 50%-60% with 4 weeks of exercise prior to inoculation ↓Lung metastases in B16F10 tumor-bearing mice	↑NK tumor infiltration ↑NK cell killing against YAC-1 and B16 cells ↑IL1 α and iNOS ↑IL6 expression ↑IL6 receptor + NK cell subset
Modulation of murine breast tumor vascularity, hypoxia and chemotherapeutic response by exercise Betof et al. 2015 PMID: 25780062	BALB/c mice with orthotopic mammary tumors \pm cyclophosphamide treatment $n = 11-12$ per group	Voluntary wheel running before and during tumor growth	↓Tumor volume with exercise alone, and greatest decrease with exercise + cyclophosphamide	↓PDGF-R expression ↑CD31 colocalization with desmin ↑VEGF expression
Modulation of blood flow, hypoxia, and vascular function in orthotopic prostate tumors during exercise McCullough et al. PMID: 24627275	Copenhagen rats with R-3327 MatLyLu prostate tumor $n = 12$ vehicle controls $n = 42$ tumor-bearing mice	Treadmill exercise, for 5 min/day for 5 days at a 10° incline and a speed of 15 meters/min	Not assessed	↑Tumor blood flow by 200% during exercise ↓Maximal vasoconstriction in response to epinephrine ↓Tumor hypoxia by 2-fold

Table 1B. Clinical publications examining associations between exercise and immune function in patients with cancer

Article	Subjects	Exercise modality	Tumor growth effects of exercise	Immune effects of exercise
Randomized controlled trial of exercise and blood immune function in postmenopausal breast cancer survivors Fairey et al. 1985 PMID: 15772062	Postmenopausal breast cancer survivors sedentary group n = 28 exercise group n = 25	Cycle ergometry sessions 3 times per week for 15 weeks	Not addressed	↑Natural killer cell cytotoxic activity
Effect of aerobic training on the host systemic milieu in patients with solid tumors: an exploratory correlative study Glass et al. 2015 PMID: 25584487	Mixed solid tumor patients sedentary group = 21 exercise group = 23	Aerobic cycle ergometry sessions 3 times per week at 55%-100% VO2 peak for 12 weeks	Not addressed	↑VO2 peak ↓Macrophage Inflammatory Protein 1 ↓VEGF No change in TNF-α ↑CD8 ⁺ and CD4 ⁺ T cells No change in NK cells
Exercise and lymphocyte activation following chemotherapy for breast cancer Hutnick et al. 2005 PMID: 16286849	Breast cancer patients recovering from chemotherapy sedentary group = 21 exercise group = 28	Treadmill or outdoor running or walking sessions for 6 month	Not addressed	↑Percentage of CD4 ⁺ CD69 ⁺ T cells (activated T lymphocytes) in blood No change in IFNγ No change in IL6
Modulation of circulating angiogenic factors and tumor biology by aerobic training in breast cancer patients receiving neoadjuvant chemotherapy Jones et al. 2013 PMID: 23842792	Women with newly diagnosed, histologically confirmed unresected stage IIB-IIIc breast adenocarcinoma Chemo alone: n = 10 Chemo + exercise: n = 10	Three supervised aerobic cycle ergometry sessions per week, 20 to 45 min/session (duration), at 55% to 100% of VO2 peak for 12 weeks	Not addressed	↑VO2 peak ↑CEP surface markers of VEGFR-2 ↑PLGF (proangiogenic factor) ↑IL8 ↓Expression of PAK4, FYB, and TNFRSF10D (converges on NF-κB pathway) in tumors

melanoma implantation in mice exhibited a 6-fold increase in NK cell infiltration into primary tumors, reduced tumor growth rate (by 60%), and halved the number of lung metastases compared with sedentary controls (42). Activity of adrenergic receptors on immune cells was key to the stimulation of NK cell activity; beta-blockers inhibited the activation of NK cells associated with exercise (42).

Tumor-associated macrophages (TAM) also contribute to innate antitumor immunity (43). Antitumor/M1 macrophages secrete proinflammatory cytokines (i.e., IFN γ and IL12), which support NK cell activation and Th1 T-cell immunity. However, in advanced cancer, TAMs differentiate to a protumor/M2 phenotype and secrete immunosuppressive cytokines such as IL10 (44).

In murine mammary carcinoma models, swimming promoted antitumor/M1 polarization of peritoneal macrophages following lipopolysaccharide (LPS) stimulation, whereas similarly treated macrophages in sedentary controls maintained a protumor/M2 phenotype (44). Additionally, chronic treadmill training increased tumor cell cytotoxicity by peritoneal macrophages by 50% (45). These data suggest that exercise-mediated shifts in macrophage polarization may increase antitumor function of TAMs.

Exercise and the adaptive immune system

In humans, large increases in the number of circulating low-, medium-, and high-differentiated T cells occur during or shortly after exercise. Exercise intensity increases the relative distribution of low-, medium-, and high-differentiated T cells with main effects seen in medium-differentiated CD4⁺ T cells and low- and medium-differentiated CD8⁺ T cells (46). These cells exhibit higher cytokine production and enhanced response to cytomegalovirus. Upon exercise cessation, circulating lymphocyte and NK cell numbers rapidly decline, even to levels below baseline, suggesting that they quickly enter back into tissues (47).

In post-chemotherapy patients with breast cancer, 12 weeks of supervised exercise did not change the mean circulating numbers of CD3⁺, CD4⁺, CD8⁺, B or NK cells, but increased the percentage of CD4⁺/CD9⁺ cells (CD9 is a marker of T-cell activation) by 50% compared with controls who did not exercise. In comparison, the percentage of CD9⁺ lymphocytes declined in the control group. *In vitro* response to lymphocyte mitogens in the exercising group was increased, compared with controls (48). These results suggest that immune competency is increased by exercise in post-chemotherapy breast cancer patients who exercise. It is not known whether this change in immune competency extends to tumor immunity, however.

To our knowledge, the role of exercise on regulatory T cells (Treg) has not been examined clinically; results in murine models have been mixed. In the MMTV-PyMT transgenic mouse mammary carcinoma, 10 weeks of voluntary wheel running decreased tumor size and caused a 4-fold reduction in CCL22 expression. CCL22 is an M2 macrophage-associated chemokine responsible for recruiting Tregs (49). There was no difference in numbers of M1 or M2 macrophages between groups, suggesting that the reduction in CCL22 is related to functional, rather than quantitative, change in M2 macrophages. A second study reported that physical activity decreased the percentage of splenic Tregs in mammary carcinoma-bearing mice (50). However, others have suggested that exercise increases Tregs (51). To

date, the impact of exercise on Treg function and distribution has not been systemically evaluated. Understanding the relationship between exercise and Treg levels with various exercise conditions could be used to increase effector T-cell/Treg ratio; the ratio of effector T cells to Tregs is associated with cancer therapy response (52–54).

Humoral Immunity and Exercise

B cells, like T and NK cells, are mobilized by exercise. Short bouts of cycling in healthy human subjects increased circulating levels of multiple B-cell subsets, with the largest proportionate increase in immature B cells. Immature B cells may redistribute to peripheral tissues for maturation and antigen detection (55). In elderly patients, 10 months of aerobic exercise, compared with flexibility and balance training, increased antibody responses to influenza vaccine (56). However, it is not known whether such effects influence tumor immunity.

Exercise-induced modulation of the tumor physiologic microenvironment and its role in immune response

To our knowledge, there are no studies that directly link exercise-induced changes in the tumor physiologic microenvironment with changes in tumor immune response. However, the literature highlighted below supports the hypothesis that modulation of the physiologic microenvironment by exercise can improve antitumor immunity.

We discuss four features: hypoxia, glucose concentration, lactate concentration, and extracellular pH (pHe). Although discussed separately, these physiologic conditions often occur simultaneously in space and time. Future exercise intervention studies should consider simultaneous measurement of these four features coincident with the evaluation of immune function.

Tumor hypoxia and innate and adaptive immune function

Most tissues possess physiologic oxygen tension above 20 mmHg (57). Whereas oxygen delivery matches metabolic demand in normal tissue, oxygen demands overpower limited supply in tumors (58). The imbalance between supply and demand leads to intratumoral hypoxia (pO₂ < 10 mmHg; refs. 58, 59). Hypoxia is prevalent in many solid cancers and contributes to chemoresistance, radioresistance, and reduced survival (6, 60, 61).

Hypoxia inhibits macrophage and NK cell activities (62, 63). Hypoxic conditions reduce the expression of NK cell surface receptor NKG2D, as well as its ligand *in vitro* (64, 65). Hypoxia impairs NK cytotoxicity of cancer cells of both hematopoietic and solid tumor origin (66). Hypoxia post-translationally upregulates the HIF1 α subunit of the transcription factor, hypoxia inducible factor-1 (HIF1), in nearly all mammalian cells, including tumor, endothelial, and stromal cells (67). Upregulation of HIF1 induces the production of VEGF, granulocyte-stimulating factors, and IL8. This in turn recruits myeloid-derived suppressor cells (MDSC) and TAMs to tumor sites (68–70). High levels of HIF1 promote myeloid cell differentiation into immunosuppressive protumor/M2 TAMs and MDSCs (71, 72).

Hypoxia also inhibits adaptive immune cell function. Hypoxia interferes with immune plasticity and disrupts the balance between effector T cells and Tregs (25, 73). Further, T-cell motility is reduced in hypoxia (74). In murine models of colorectal

carcinoma, hypoxia reduced differentiation of CD4⁺ T cells by 20% to 40%, while enhancing the number and function of Tregs (75). Hypoxic effector T cells exhibit decreased IFN γ and IL2 production (62).

Although there are many studies investigating T-cell function in the context of the hypoxic tumor microenvironment, few have examined B-cell function under hypoxic conditions. Studies indicate that hypoxia and oxygen gradients vary in lymphoid tissues, which could impact B-cell function (76–79). Germinal centers in lymph nodes and spleen are important for B-cell maturation. Germinal centers are hypoxic with high levels of HIF1 α . Thus, B cells encounter varying conditions of hypoxia as they migrate to and from lymphoid organs into circulation. It is plausible that hypoxia controls B-cell migration, differentiation/function, and activation in response to antigens as well as tolerance (76–79). Lee and colleagues identified B cells as key immune cells in pancreatic cancer progression (80). In a mouse model of pancreatic ductal adenocarcinoma (PDAC), they demonstrate the importance of hypoxia and stabilization of HIF1 α . They show that pancreas-specific *Hif1a* deletion promotes PDAC initiation with a concurrent increase of B cells in the pancreas, whereas B-cell depletion suppresses pancreatic cancer progression (80).

Key mechanisms underlying hypoxic immunosuppressive effects include (i) signaling through adenosine receptors (A2A adenosine receptors), (ii) desensitization of chemokine receptors, (iii) downregulation of major histocompatibility complex (MHC) class I molecules on tumor cells (81, 82), and (v) recruitment of immunosuppressive cells into the tumor microenvironment. Hypoxia-induced inhibitory effects may be reversible, however. Supplemental oxygen breathing revives effector immune responses. Housing tumor-bearing mice at hyperoxic conditions (60% O₂) reversed hypoxia-driven adenosinergic action, enhanced tumor infiltration by CD8⁺ T cells by 3-fold, and shifted cytokine production toward immunostimulatory cytokines (IFN γ ; ref. 81).

Exercise modulates tumor hypoxia

Several studies show that exercise reduces tumor hypoxia. In an orthotopic rat prostate cancer model, acute exercise increased tumor blood flow 2-fold, thereby increasing O₂ delivery to tumors. Tumor hypoxic fraction was reduced by up to 15% (14, 16). Treadmill running increased microvessel density, promoted vessel maturity, and reduced hypoxic tumor fraction compared with sedentary controls (12, 13, 16). Chronic voluntary wheel running increased microvessel density by 50%, tripled the area of pericyte-covered vasculature, and halved hypoxic fraction (12) in mice bearing the 4T1 tumor. Schadler and colleagues demonstrated that increased vascular maturity associated with exercise was related to activation of calcineurin-NFAT_TSP-1 signaling induced by increased intravascular shear stress (16). In theory, reduced tumor hypoxia should destabilize HIF1 α expression. Interestingly, however, Jones and colleagues showed increased HIF1 α in the MDA-MB-231 xenograft with exercise, despite decreases in hypoxia (13). These findings suggest that exercise alters intratumoral HIF1 α expression in ways independent of the improved oxygenation level. The fact that HIF1 levels can be upregulated in some tumors, regardless of the oxygenation status, leads to a cautionary note about the downstream effects of exercise on tumor growth. More studies are required to understand these implications.

Exercise and glucose deprivation

The high rate of glucose consumption in tumors, and deficiencies in glucose delivery by dysfunctional tumor vasculature, can lead to tumor subregions with near-zero glucose concentrations, even in nonnecrotic regions (83). Viable tumor cells residing in hypoglycotic (low glucose concentration) regions likely rely on other substrates to maintain viability, such as glutamine or fatty acids (84). Glucose availability is essential for effective immune function, because activated immune cells rely on glycolysis to produce precursors for cell division (85). Thus, one might expect that immune function would be inhibited in hypoglycotic tumor subregions. The effects of exercise on glucose metabolism in immune cells are understudied, however.

Glass and colleagues reported on the effects of daily treadmill exercise in three claudin-low murine tumor models (86). "Claudin-low" represents breast tumors that express genomic markers of dedifferentiation (87). Exercise inhibited growth compared with sedentary controls in one tumor line but stimulated growth in a second one. The tumor line that showed an accelerated growth rate with exercise had upregulated HIF1 levels. HIF1 is a master transcriptional regulator of glycolysis (88). Glycolysis is associated with accelerated tumor growth, because it generates precursors necessary for cell division (88). Corroborating the HIF1 result, metabolomic analysis revealed that glycolysis was upregulated by exercise in the growth-accelerated tumor. Exercise slowed tumor growth in three of six colorectal cancer patient-derived xenograft (PDX) tumors, whereas it exerted no effect on tumor growth in three others (89). The growth-inhibited lines showed metabolomic changes consistent with reduced mitochondrial metabolism. It is unknown whether exercise affects the nature of glucose consumption in tumor-associated immune cells and, if so, how that might affect the immune function.

Lactate metabolism

Tumor cells exist in an acidic microenvironment as a result of either anaerobic or aerobic glycolysis. In hypoxic conditions, cells must use glycolysis to generate energy (90). Aerobic glycolysis produces precursors for DNA and lipid synthesis (91). Lactic acid is the end-product of glycolysis, expelled from cells via monocarboxylic acid transporters (MCT; ref. 92). Acidity is created mainly by lactic acid via glycolysis (93). Although lactate was traditionally thought to be a glycolytic waste product, it is now established that lactate can be consumed by aerobic tumor cells; alanine and glutamate are primary catabolites (94). Consumption of lactate by aerobic tumor cells reserves glucose for hypoxic tumor cells deeper within the tumor (90). The sharing of energy substrates between aerobic and hypoxic tumor cells is a key mechanism for hypoxic tumor cell survival (90). It is not known whether immune cells can catabolize lactate.

Lactate, acidosis, pH, and immune response

In contrast to normal tissues, which possess an extracellular pH (pHe) of about 7.5, the median pHe in solid tumors is 6.8–7.0 (95, 96). Elevated lactate and the associated low tumor pHe impair lymphocyte cytotoxicity, chemotaxis, cellular respiration, and proliferation (97). At pHe < 6.5, *in vitro* random leukocyte motility is greatly decreased (98). At pHe < 6.7, lymphocyte and NK cell cytotoxic activities against leukemia target cells are approximately halved (99, 100). Similarly, NK cells cultured with

10 to 15 mmol/L lactate, a physiologic tumor lactate range (101, 102), exhibit 5-fold reduction in target cell cytotoxicity (103).

Immunosuppression in acidic environments may partially be a consequence of compensatory shifts in T-cell metabolism (104), as activated T cells are particularly reliant on glycolysis (105). High lactate generated by tumor cells (106) disturbs the gradient that drives T-cell lactate efflux, because MCT1 facilitates lactate transport out of cells in a manner that is dependent upon the concentration gradient across the cell wall (107). Increased intracellular lactate suppresses T-cell metabolism and function (105).

Exercise and tumor lactate/acidity

Aerobic exercise normalizes the immunosuppressive, acidic tumor microenvironment. Treadmill running in mice with mammary carcinomas reduced tumor and circulating lactate concentrations by approximately 17% compared with sedentary counterparts (108). In sarcoma bearing rats (109), treadmill running decreased glucose conversion to lactate by approximately 50% in peripheral macrophages and lymphocytes. The reduction in tumor lactate production was accompanied by a 2-fold increase in peripheral macrophage phagocytic activity and a 75% increase in peripheral lymphocyte proliferation (measured *in vitro*). Although changes in the profile of peripheral immune cells in response to exercise do not necessarily reflect changes in the tumor, the study, nevertheless, demonstrates that reducing lactate exposure potentiates the immune function.

Future Directions and Conclusion

In this review, we explore the hypothesis that exercise modulates the tumor physiologic microenvironment and, consequently, influences immune function and activity. The current literature supports the concept that well-oxygenated, less acidic environments (i) improve the function of T cells and NK cells; (ii) promote antitumor activity in TAMs; and (iii) reduce expression of some immune checkpoints. Additional preclinical and clinical trials of exercise should be conducted in which immune function is studied in the context of the tumor physiologic microenvironment, with particular attention paid to hypoxia, glucose concentration, lactate concentration, and extracellular pH (pHe).

More work is required to establish how physiologic effects of exercise modulate the tumor microenvironment. Corroborative measurements of circulating and intratumoral immune cells may help to clarify the link between tumor microenvironment changes and changes in immune function/activity. Additionally, metabolic profiling of immune cells isolated from tumors of exercising and sedentary subjects would shed light on the metabolic adaptations in an exercise-primed tumor microenvironment. In order to be interpretable, preclinical and clinical studies of exercise require carefully defined and controlled exercise regimens. Preclinical models of exercise in tumor-bearing animals can shed light on underlying mechanisms and help to optimize combinations of exercise with other therapeutic approaches.

Rigorous studies that elucidate the link between exercise and immune cell function in the tumor microenvironment and in the periphery will also serve as a guide of how to implement exercise in the context of immunotherapies that harness the immune system against cancer. Successful immunotherapy relies upon the ability of innate and adaptive immune cells,

such as macrophages, NK cells and T cells, to infiltrate the tumor parenchyma and eliminate tumor cells. The factors in the tumor physiologic microenvironment that inhibit the penetration and function of host immune cells will likely also interfere with immunotherapies. To elaborate further, two examples are provided below:

1. CAR T cells are engineered to express chimeric antigen receptors (CAR) that specifically target and eliminate tumor cells, independent of the MHC (110). Because MHC expression is downregulated by hypoxia (81, 82), CAR T cells have an advantage over host T cells that rely on the MHC-based recognition of tumor cells in the hypoxic tumor microenvironment. However, T-cell (including CAR T-cell) trafficking to the tumor via the vasculature and penetration into the tumor bed are inhibited by hypoxia. In other words, the T cell has to reach tumor cells before it can kill them. Thus, the tumor physiologic microenvironment, and not the MHC, is the gatekeeper for effective T-cell infiltration into the tumor bed. As discussed above, high lactate concentrations and low pH can interfere with the ability of T cells to kill tumor cells. These effects are not reliant on MHC expression. It is important to note that CARs have been effective in treating certain types of lymphomas (111, 112). The physiologic microenvironment of lymphomas or blood cancers may be more permissive toward an effective immune function. For example, lymphomas are only mildly hypoxic (113). However, results regarding lactate levels are mixed. Elevated lactate levels are not common in CNS lymphomas (114). Further, there is some evidence that lymphomas are more reliant on oxidative phosphorylation than glycolysis (115), which would reduce lactate concentrations. However, elevated lactate concentrations and lowered pHe have been observed in preclinical lymphoma models (116, 117). Compared with normals, blood lactate concentrations are relatively high in dogs with non-Hodgkin lymphoma (118). These data suggest that lactate levels may be relatively normal in some lymphomas, but not all. If lymphomas are relatively oxic, then the physiologic microenvironment would be permissive to enhanced CAR T-cell function; however, lactate levels may counterbalance the positive effects of normoxia. The effects of elevated lactate on the CAR T-cell immune function are relatively unexplored in lymphomas. Further, the role that exercise may play in the lymphoma physiologic microenvironment is not defined.
2. Immune-checkpoint inhibitors are antibodies that bind immune receptors to inhibit T-cell function. T-cell function is inhibited when PD-1 on T cells interacts with its ligand, PD-L1, on tumor cells or myeloid immune cells. PD-L1 is regulated by HIF1, so in situations where exercise reduces hypoxia and its dependent transcription factor, HIF1, one would expect decreased PD-L1 expression (119–121). Reduced PD-L1 would reduce PD-L1/PD-1 interaction and improve T-cell function within the tumor. It is unknown whether T-cell function would be improved in conditions where hypoxia is lowered but lactate levels remain the same. Thus, correction of hypoxia by exercise may be insufficient to restore immune cell function if lactate concentrations remain elevated as a result of aerobic glycolysis.

It is clear that additional studies are required to resolve the effects of exercise on the physiologic microenvironment. Parallel studies examining the function of innate and adaptive immunity in the microenvironment, as influenced by exercise, are necessary to decipher the full potential of this therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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