Regulation of immune cell trafficking by febrile temperatures

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ABSTRACT
Fever is a complex physiological response to pathogen infection and injury. One of the beneficial effects of febrile temperatures is stimulation of immune cell trafficking to the lymphoid organs and inflamed tissues, thereby enhancing immune surveillance during infection and inflammation. This trafficking process consists of a highly ordered adhesion cascade that includes tethering and rolling of immune cells along the vessel walls, chemokine-induced activation, firm arrest and diapedesis. In this review, we summarize the current findings of how febrile temperatures regulate the immune cell trafficking process. Febrile temperatures play multiple roles in the functional regulation of critical biomolecules involved in each step of the ordered adhesion cascade that includes L-selectin, chemokines, and α4 and β2 integrins. A better understanding of febrile temperature-induced regulation of immune cell trafficking will shed light on modulating the immunity to fight against infection and inflammation.

1. Introduction
Traffic ing of immune cells from blood circulation to lymphoid organs and inflamed tissues has a crucial role in immune surveillance and host defense [1–3]. The entry of blood-borne naive lymphocytes to lymph nodes preferentially occurs at high endothelial venules (HEVs), and this process is essential for lymphocytes to encounter antigens and antigen-presenting cells, such as dendritic cells [4]. HEVs are composed of plump endothelial cells that bulge into the vascular lumen. Recirculation of antigen-specific lymphocytes through lymph nodes allows them to survey their target antigens in any part of the body. This course of action provides effective immune surveillance against foreign invaders (such as viruses, bacteria, and helminths) and alterations in the body's own cells (such as abnormal self-antigens in cancer) [2]. During homeostasis, HEVs are found only in the lymphoid organs, but they can develop in non-lymphoid tissues during chronic inflammatory diseases and cancer; thus, they are associated with high levels of lymphocyte infiltration into these tissues [5–7]. Furthermore, the recruitment of neutrophils, monocytes, and some other immune cells to inflamed tissues also requires adhesion to and transmigration through the blood vessel walls [8,9].

Febrile temperature is defined as a body temperature above the normal range, which can result from hyperthermia or fever [4,10]. Hyperthermia is a condition in which an individual's body produces or absorbs more heat than it dissipates due to failed thermoregulation while the body temperature set-point remains normal [11], whereas fever is a complex physiological response to infection or injury, and the set-point is elevated through cytokine-mediated changes in the hypothalamic regulation [12–14]. Local release of endogenous prostaglandin E2 (PGE2) and pyrogenic cytokines, including tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), and interleukin-6 (IL-6) act systemically to induce fever [4,15]. An increase of 1–4°C in core body temperature is associated with improved organism survival and the resolution of many infections [4]. During the last two decades, emerging evidence has shown that febrile temperatures can enhance immune cell trafficking [16,17]. In this review, we summarize and discuss the current understanding of how febrile temperatures regulate the immune cell trafficking process.

2. Adhesion cascade during immune cell trafficking
The immune cell trafficking process consists of a highly ordered adhesion cascade that includes tethering and rolling of immune cells along the HEV walls, chemokine-induced activation, firm arrest, and transendothelial migration [3,18,19].

i. The initial tethering and rolling of lymphocytes are mainly mediated by the interaction between selectins and their ligands. L-selectin (also known as CD62L), expressed by leukocytes, recognizes its counter-receptor (peripheral node addressin, PNAd) on the HEVs to mediate the tethering and rolling [2]. In addition, inactive α4β1, α4β7, and αLβ2 integrins are also able to support...
lymphocyte rolling by binding to their endothelial ligands, vascular cell adhesion molecule 1 (VCAM-1), mucosal vascular addressin cell adhesion molecule 1 (MAdCAM-1), and intercellular adhesion molecules (ICAMs) [8,20], respectively.

ii. Chemokine-induced activation is the critical step to switch from the rolling to firm arrest [21]. The homeostatically expressed chemokine ligands CC-chemokine ligand 21 (CCL21), CXC-chemokine ligand 12 (CXCL12), and CXCL13 are the crucial factors for lymphocyte extravasation through the HEVs in lymph nodes. Naïve T lymphocytes express CC-chemokine receptor 7 (CCR7) and CXC-chemokine receptor 4 (CXCR4), which are the receptors for CCL21 and CXCL12, respectively. Furthermore, naïve B lymphocytes express CXCR5, which is the receptor for CXCL13, in addition to CCR7 and CXCR4 [3,22,23]. During this process, chemokines activate integrins by rapidly triggering an inside-out signaling network that regulates the binding of intracellular effector proteins (e.g., talin and kindlin) to the cytoplasmic domains of integrins [24,25]. Binding of the effector proteins converts the inactive integrin in the low-affinity (bent) conformation into its active form, characterized by an extended conformation with high affinity for the ligands [26,27].

iii. Activated α4 and β2 integrins (e.g., α4β1, α4β7, αLβ2, and αMβ2) mediate firm cell arrest on the endothelium by binding to their distinct endothelial ligands (VCAM-1, MAdCAM-1, and ICAM-1) with high affinity.

iv. The final transmigration step across HEVs involves adhesion molecules, including α4β1, αLβ2, VCAM-1, ICAM-1, ICAM-2, platelet endothelial cell adhesion molecule-1 (PECAM-1), junctional adhesion molecule 1 (JAM-1), and JAM-2 [28,29].

3. Febrile temperatures amplify L-selectin-dependent lymphocyte adhesion and trafficking

By binding to PNAd, L-selectin mediates the initial tethering and rolling of immune cells along the vessel walls. Treatment of lymphocytes with the fever-range temperatures (38–41°C) strongly increases L-selectin-dependent lymphocyte adhesion to cryosections of lymph node HEVs in vitro and trafficking to peripheral lymph nodes (PLNs), mesenteric lymph nodes (MLNs), and Peyer’s patches (PPs) in short-term homing assay [17,30]. Moreover, exposure of mice to fever-range whole-body hyperthermia (WBH) or LPS/turpentine-induced fever stimulates lymphocyte homing to secondary lymphoid tissues in an L-selectin-dependent manner [17].

Investigation of the mechanisms underlying the thermal regulation of L-selectin adhesion shows that fever-range hyperthermia neither increases L-selectin surface density on lymphocytes nor alters the expression of PNAd on HEVs [30,31]. Further study demonstrates that IL-6 cooperates with a soluble form of IL-6 receptor-α (sIL-6Rα) and the membrane-anchored gp130 to amplify L-selectin adhesion in response to thermal stimulation [32,33]. The MEK1-ERK1/2 signaling pathway acts as the downstream of gp130-linked IL-6/sIL-6Rα trans-signaling to increase L-selectin/cytoskeleton interactions and L-selectin avidity/affinity in lymphocytes [4,32]. Furthermore, 11 amino acids on the C-terminus of L-selectin are essential to mediate the association with the cytoskeleton [34].

4. Febrile temperatures enhance endothelial expression of chemokine CCL21

The interaction between chemokine CCL21 displayed on the lumenal surface of HEVs with Gα13-protein-coupled chemokine receptor CCR7 on lymphocytes triggers integrin activation and the subsequent firm cell arrest [1,3,22,23]. Indeed, fever-range WBH treatment substantially increases the intravascular presentation of CCL21 without affecting the weak-to-nondetectable staining for other homeostatic chemokines (CXCL12 and CXCL13) on HEVs [31]. However, there is no change in CCR7 expression on T lymphocytes after thermal stress in vitro and in vivo. This observation excludes a role for increased expression of chemokine receptors in enhancing T lymphocyte homing after thermal stress [35].

5. Febrile temperatures regulate integrin-mediated lymphocyte adhesion and transmigration

α4 and β2 integrins involve in all the steps during lymphocyte homing and thus have essential roles in regulating lymphocyte trafficking to the lymphoid organs and inflamed tissues. The expression of α4 integrin ligands (VCAM-1 and MAdCAM-1) and β2 integrin ligands (ICAMs) on the blood vessels is homeostatic and also inducible upon inflammation [9,36]. Emerging evidence shows that febrile temperatures regulate α4- and β2-integrin-mediated lymphocyte adhesion and transmigration via distinct mechanisms.

Fever-range hyperthermia treatment (40°C, 12 h) significantly enhances α4β7 integrin-dependent adhesion of murine TKI lymphoma cells and human peripheral blood lymphocytes (PBLs) to HEV cryosections in vitro [37]. Similarly, fever-range WBH treatment of mice also causes α4β7 integrin-dependent lymphocyte redistribution in lymphoid tissues [17], without affecting the expression of α4β7 integrin on lymphocytes [17,37] or MAdCAM-1/VCAM-1 on HEVs [31,35].

A recent study reveals a mechanism underlying the functional regulation of α4 integrin using fever-range hyperthermia treatment and an infection-induced mouse model of fever [35]. Febrile temperatures (≥38.5°C) can efficiently enhance the expression of heat shock protein 90 (Hsp90) in T lymphocytes [35,38]. Hsp90 binds to the cytoplasmic tail of α4 and induces association of talin and kindlin-3 with the cytoplasmic tails of integrin β subunit, triggering α4 integrin activation via inside-out signaling. Moreover, the N- and C-terminus of one Hsp90 molecule can simultaneously bind to two α4 tails, resulting in dimerization and clustering of α4 integrins on the plasma membrane and subsequent activation of the FAK-RhoA signaling pathway in T lymphocytes, thereby promoting T lymphocyte adhesion and transmigration. This regulation of α4 integrin function does not require
the ATPase activity of Hsp90, suggesting that this function is distinct from Hsp90’s chaperone function, which requires the energy released from ATP hydrolysis.

Abolishing Hsp90-α4 interaction in vivo inhibits WBH-induced T lymphocyte trafficking to draining lymph nodes [35]. Moreover, in Salmonella typhimurium infection-induced mouse model of fever [39], disruption of Hsp90-α4 interaction in the mice markedly decreases the number of infiltrated T lymphocytes and increases bacterial dissemination in the small intestine 5 days after the oral administration of S. typhimurium [35]. Thus, Hsp90-α4 integrin axis is a thermal sensory pathway that promotes T lymphocyte trafficking to inflamed tissues and facilitates the clearance of bacterial infection. It is noteworthy that fever-induced Hsp90 expression in T lymphocytes can last for at least 48 h, which enables a persistent regulation of α4 integrin function even after the temperature drops back to the normal range [35]. In addition to T lymphocytes, Hsp90-α4 integrin axis can also enhance the trafficking of other α4 integrin-expressing immune cells, such as monocytes, and B lymphocytes [35].

ICAM-1 and ICAM-2 are two major ligands for β2 integrins during steady-state trafficking of lymphocytes [40,41]. Although febrile temperatures do not increase the surface density and ligand-binding affinity of integrin αLβ2 [30,37], fever-range thermal stress has been shown to enhance endothelial expression of ICAM-1 [31,42–44]. In WBH-treated mice, the ICAM-1 expression on HEVs is strongly upregulated via an IL-6 trans-signaling pathway to facilitate αLβ2-integrin-dependent lymphocyte adhesion to and transmigration across the HEV walls [31]. Conversely, the intravascular density of ICAM-2 is not altered by thermal stress. Thus, the ”HEV axis” works as a thermally sensitive alert system by amplifying ICAM-1 density to promote β2-integrin-dependent lymphocyte trafficking to the lymphoid organs [31,45].

6. Perspective
i. In this review, we summarize the mechanisms underlying the regulation of immune cell trafficking by febrile temperatures caused by hyperthermia or fever. It is noteworthy that hyperthermia is different from fever. For example, peripheral blood vessels constrict during fever, whereby the body heat is conserved, whereas they dilate to dissipate the heat in hyperthermia [46–48]. This difference may prompt a major revision of the mechanisms underlying the regulation of immune cell trafficking.

ii. The regulation of immune cell trafficking by thermal stress is explained by the nature of the adhesion molecules on both immune cells and HEVs. Febrile temperatures regulate each step of the adhesion cascade differently (Figure 1). Firstly, L-selectin-dependent lymphocyte tethering and rolling are amplified in a gp130-linked IL-6/sIL-6Rα trans-signaling pathway. Secondly, fever promotes α4-positive immune cell adhesion and transmigration via a thermal sensory pathway comprising Hsp90-α4 integrins. By the binding of
Hsp90, α4 integrins are activated via inside-out signaling. Meanwhile, α4 integrin dimerization and clustering is induced on the cell membrane to activate the FAK-RhoA signaling pathway. Thirdly, febrile temperatures “preferentially” enhance the intravascular density of two molecules involved in homeostatic trafficking (CCL21 and ICAM-1). These two molecules act cooperatively to optimize the binding activity of αLβ2 integrin. In addition, thermal induction of ICAM-1 involves an IL-6 trans-signaling pathway. Taken together, febrile temperatures systemically regulate immune cell delivery to HEVs, thereby promoting immune surveillance during infection and inflammation.

iii. Hsps are cytoprotective proteins that are constitutively expressed and also rapidly induced under proteotoxic stress conditions such as heat [4,49]. Although Hsps were originally discovered in the context of heat shock (42–45 °C), they are also induced by febrile temperatures (38–41 °C) in mammalian cells [49,50]. Multiple lines of evidence have demonstrated that Hsps, especially Hsp70 and Hsp90, are involved in the febrile temperature-induced regulation of both innate and adaptive immunity [4,51–53]. However, the direct participation of Hsps in immune cell trafficking is poorly understood. A recent study shows that fever promotes T lymphocyte trafficking via a thermal sensory Hsp90-α4 integrin pathway [35]. By inducing selective binding of Hsp90 to α4 integrins, but not β2 integrins, fever increases α4-integrin-mediated T lymphocyte adhesion and transmigration. Additionally, another study finds that Hsp70 could associate with the cytoplasmic domain of integrin β7 subunit [54]. Furthermore, Hsp40, Hsp60, and Hsp70 bind to both α4 and β2 integrins [35], suggesting that these Hsps may involve in the constructive regulation of integrin-mediated cell adhesion and migration by thermal stress, which needs further investigation.

iv. Immune cell trafficking participates in all kinds of autoimmune diseases (such as multiple sclerosis, inflammatory bowel disease, lupus, rheumatoid arthritis, etc.) and even cancer. Based on the mechanisms discussed in this review, it might be possible to promote immune cell trafficking to enhance the immune response against infections and cancer, or suppress it during chronic inflammation and autoimmune disorders. Thereby, modulation of immune cell trafficking may help developing disease management strategies [55–57].

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