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Fine-Tuning Immune Surveillance by Fever-Range Thermal Stress

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Abstract

An effectively orchestrated immune response to infection and disease depends on efficient trafficking of lymphocytes across vascular beds at distinct tissue sites. Local inflammation and systemic fever increase immune surveillance to immune-relevant sites throughout the body. During the initiation phase of inflammation, this tightly-regulated process improves leukocyte trafficking to the secondary lymphoid organs where they undergo activation and expansion in response to cognate antigen. In the resolution phase following the clearance of the invading pathogen, lymphocyte entry is rapidly returned to baseline conditions. Specialized blood vessels termed high endothelial venules (HEVs) have emerged as critical ‘hotspots’ controlling the rate of lymphocyte entry into lymphoid organs during both phases of inflammation. In this review we will examine the remarkably tight regulation of lymphocyte trafficking across HEVs conferred by inflammatory cues associated with the thermal element of fever. These studies have revealed a novel role for interleukin-6 (IL-6) trans-signaling in eliciting systemic effects on lymphocyte trafficking patterns to fine-tune immune surveillance.

Life in the fast lane: contributions of lymphocyte trafficking to adaptive immunity

Immune protection from infection and disease is dependent on the ability of lymphocytes to efficiently traffic across distinct vascular sites throughout the body. Innate immune cells engulf invading pathogens and mature, releasing inflammatory mediators that cause local inflammation and systemic fever. The ensuing increase in lymphocyte trafficking triggered by inflammatory cues is now appreciated to be a lynchpin in the process that heightens immune surveillance (Fig. 1) [1–4]. Following the clearance of the invading pathogen the inflammatory response enters a tightly-regulated resolution phase in which leukocyte trafficking rapidly returns to baseline steady-state conditions.

To mount an effective immune response to infection, individual cellular components of the adaptive immune system (e.g., CD8 T cells, CD4 T cells, and B cells) must first become activated [1–4]. The adaptive immune system must overcome monumental logistical problems since under non-challenge conditions only 1 in 10^5 – 10^6 T cells are able to recognize a given antigenic determinant [5,6]. To ensure that these rare antigen-specific T cells encounter antigen-presenting cells (APC) carrying cognate antigens, the disparate cell types of the adaptive immune system are brought together in the secondary lymphoid organs, i.e., peripheral lymph nodes, mesenteric lymph nodes and Peyer patches [1–4,7–9]. These organs provide a nurturing environment for lymphocyte education and activation and are strategically positioned to provide the first line of defense against invading pathogens that enter the host through the skin, lungs, gastrointestinal or genitourinary systems. It is estimated in mice that blood-borne

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lymphocytes continuously enter lymph nodes at a rate of $\sim 1.25 \times 10^5$ cells per hour [10]. As a result of this high throughput screening process, the lymphocyte content of an individual lymph node turns over ~ 3 times a day.

The major gateways for lymphocyte entry into secondary lymphoid organs are specialized blood vessels termed high endothelial venules (HEVs) [1–4,7–9]. These post-capillary venules reside exclusively in the paracortical region of lymphoid organs under non-inflammatory conditions. In contrast to resting squamous endothelium in extralymphoid organs which supports only a low rate of lymphocyte trafficking, HEVs lined by cuboidal endothelial cells are sites of highly efficient lymphocyte trafficking under homeostatic conditions. Multiple discrete molecular interactions confer tight regulation over which lymphocyte subsets can traffic across distinct vascular endothelial beds to gain entry to underlying tissues. These molecules participate in a multistep adhesion cascade required for migration across vascular endothelium that includes: (1) primary tethering and rolling of lymphocytes along vessel walls, (2) activation of chemokine receptors on lymphocytes by chemokines presented on the vascular endothelium, (3) secondary firm arrest of lymphocytes on vessels, and (4) extravasation of lymphocytes across the vascular endothelial barrier (Fig. 2) [1,3,4,7–9]. While the mechanisms governing leukocyte trafficking in HEVs of lymphoid organs under homeostatic conditions have been carefully elucidated, the effects of inflammatory activation of HEVs on leukocyte trafficking during the initiation and resolution phases of immunity continue to be an area of intense investigation.

Several lines of evidence point to HEVs being critical checkpoints in the development of the adaptive immune response. One major effect of local inflammation on lymphoid organs is that gatekeeper HEVs allow the trafficking of leukocyte subsets which are normally excluded from peripheral lymphoid organs, such as monocytes, natural killer (NK) cells, and effector CD4 or CD8 T cell subsets. Local inflammation in response to activated dendritic cells allows NK cells and effector memory CD4 T cells to efficiently interact with inflamed HEVs [11–13]. NK cells and monocytes are recruited to inflamed lymph nodes by the increased presentation of chemokines such as CXCL9 and 10 on HEVs [13,14]. Effector and memory CD4 T cells gain entry through a CXCL9 and 10-independent mechanism mediated by increased expression of the adhesion molecule P-selectin [11]. Collectively, these leukocytes license antigen-presenting cells to prime T cell activation and support the ensuing development of the immunoprotective response [11–14]. Inflammation in response to antigen challenge also induces the intravascular display of the inflammatory chemokine CCL2 on HEVs, allowing monocytes to traffic across HEVs through a mechanism dependent on CCL2/CCR2 interactions [3,15]. Blood-borne monocyte-derived antigen-presenting cells have recently been shown to use CCR2, and as-yet unidentified chemokine ligands, to traffic through the HEVs of inflamed lymph nodes where they drive strong Th1 polarized immune responses [16].

The increased vascular presentation of CXCL9 and 10 during inflammatory responses has also been suggested to assist in the resolution phase of the immune response by allowing effector CD8 T cells to efficiently interact with inflamed HEVs [17]. These effector T cells enter the lymph node and kill antigen-presenting cells, thus terminating T cell activation and expansion. Collectively, these findings suggest that inflammation of HEVs is central to orchestrating an effective immune response in which the trafficking patterns of distinct leukocyte subsets are altered in lymphoid organs.

Inflammation also supports increased trafficking of naïve lymphocytes across HEVs of secondary lymphoid organs, thereby increasing the probability that rare T cells restricted to specific antigens will encounter and be activated by antigen-presenting cells. Multiple mechanisms contribute to the increase in naïve T cell trafficking observed in draining lymph nodes following local inflammation. Inflammation triggered by viral infection remodels the

feeding arterioles that supply HEVs, enhancing the circulation of lymphocytes into responding nodes [18]. Additionally, both viral infection and local inflammation in response to the injection of activated dendritic cells cause an increase in the total number of HEVs in enlarged inflamed lymph nodes, providing more gateways for naïve lymphocytes [18–20]. These inflammatory stimuli cause normally static HEVs to proliferate through a mechanism dependent on VEGF signaling [19,20].

A unifying feature of the inflammatory mechanisms described above is that they are driven by local inflammatory stimuli and are restricted to HEVs in lymph nodes that drain infected tissues or sites of antigen challenge. While these draining lymph nodes are the first line of defense against invading pathogens, systemic inflammatory cues may play an important role in preventing the dissemination of disease. Until recently there was little known about the molecular changes in the expression of trafficking molecules on HEVs that are required to mobilize trafficking of naïve lymphocytes during acute inflammation. This review will focus on insights gained from studying the thermal element of fever as a model of an acute inflammatory response. This inflammatory cue fine-tunes immune surveillance by creating dynamic changes in lymphocyte trafficking patterns in HEVs of lymphoid organs during the initiation and resolution phases of inflammation [21–30].

Immune ‘hot spots’: HEVs as unique vascular targets of the thermal element of fever

Fever is a highly evolutionarily conserved cardinal feature of the inflammatory response. It has been documented in both endotherms and ectotherms and is linked to improved survival during infection [26,31–33]. In recent years considerable progress has been made in understanding the molecular mechanisms underlying the protective action of fever in the context of lymphocyte trafficking. These studies employed a reductionist approach to examine the thermal component of fever by elevating the core body temperatures of experimental subjects to the fever-range (39.5–40°C) for prolonged periods (≤6 hours). [34–38]. Fever-range thermal stress augments host immune surveillance by tightly regulating the intravascular adhesion events that govern lymphocyte trafficking across HEVs of lymphoid organs [21–30].

An early indication that lymphocyte trafficking might be affected by thermal stress came from observations that febrile temperatures acutely change the tissue distribution of lymphocytes. Thermal stress in both the fever-range (39–40°C) and heat shock-range (41.5–42°C) causes a transient decrease in the number of lymphocytes in the peripheral blood compartment in mice as well as in humans [21,35,37,39–41]. In mice, this decrease in the number of circulating lymphocytes was accompanied by an ~2-fold increase in trafficking of naïve and central memory T cells across HEVs of secondary lymphoid organs [21,27–30]. Migration across vessels at other tissue sites is not affected by thermal stress. The change in HEVs represents a profound influence on an already highly efficient trafficking mechanism. Heightened trafficking of naïve lymphocytes across HEVs in lymph nodes and Peyer patches during febrile responses favors the chance that rare T lymphocyte clones are activated by cognate antigens presented on dendritic cells.

One of the remarkable aspects of fever-range thermal stress is that it acts on distinct cell types to regulate each step in the highly integrated adhesion cascade required for lymphocyte extravasation across HEVs (Fig. 2). The L-selectin homing receptor mediates primary tethering and rolling of T lymphocytes (step 1) via engagement of sialomucin-like endothelial molecules collectively termed ‘peripheral lymph node addressin’ (PNAd) that are expressed exclusively on the luminal surface of lymph node HEVs (Fig. 2) [3,7,42]. Thermal stress acts directly on lymphocytes, increasing the avidity and/or affinity of L-selectin for PNAd, without altering its

expression, density, topographic localization, or N-terminal lectin binding activity [22–25]. The increase in L-selectin binding activity is dependent on 11 amino acids of the cytoplasmic tail which constitutively interact with the cytoskeletal linker protein α -actinin [22,24,43]. Fever-range thermal stress causes L-selectin to stably associate with the detergent-insoluble cytoskeletal matrix [22,24]. Stable associations with cytoskeletal scaffolding are proposed to enhance L-selectin tensile strength, thereby improving the initial tethering and rolling of lymphocytes along the luminal surface of HEVs. Fever-range thermal stress similarly increases the binding activity (but not expression) of another lymphocyte tethering/rolling molecule, $\alpha 4\beta 7$ integrin, allowing for improved interactions with its endothelial ligand mucosal addressin cell adhesion molecule-1 (MAdCAM-1) on the HEVs of mesenteric lymph nodes and Peyer patches [25]. In sharp contrast, fever-range thermal stress fails to stimulate $\alpha 4\beta 7$ integrin-mediated adhesion of lymphocytes to the extracellular matrix ligand, fibronectin [25]. These findings suggest that fever-range thermal stress exerts highly specific effects on distinct, but overlapping epitopes involved in $\alpha 4\beta 7$ integrin binding to cellular (MAdCAM-1) and non-cellular (fibronectin) ligands [44–47].

Fever-range thermal stress also acts independently on the vascular endothelium in HEVs, regulating the transition of lymphocytes from primary rolling interactions to secondary firm arrest. Engagement of the chemokine CCL21, which is presented on the glycocalyx of HEVs in secondary lymphoid organs, by the $G\alpha_1$ protein-coupled CCR7 receptor on lymphocytes (step 2) enables lymphocytes to transition from primary to secondary adhesion (Fig. 2) [3,7, 8]. CCL21 is generally considered to be a constitutively expressed homeostatic chemokine in HEVs. Thus, it came as a surprise when it was determined that fever-range thermal stress augments the luminal display of CCL21 on HEVs [28].

In response to chemokine activation, the $\beta 2$ integrin leukocyte-function associated antigen-1 (LFA-1) is activated by inside-out signaling that results in ligand-independent conformational changes in LFA-1 that increase its affinity for its endothelial ligands intercellular adhesion molecules-1 and 2 (ICAM-1 and 2). Under steady-state conditions ICAM-1 and 2 have redundant functions in supporting firm arrest (step 3) and transendothelial migration (step 4) of lymphocytes in HEVs (Fig. 2) [28,30]. Fever-range thermal stress tilts the balance toward a predominant role for ICAM-1 as a gatekeeper arrest molecule in HEVs. In this regard, febrile temperatures cause a substantial increase in the intravascular density of ICAM-1, thereby improving both firm arrest and transendothelial migration of naïve lymphocytes (Fig. 3) [28, 30,48,49]. ICAM-2 expression is conversely not altered by thermal stress in HEVs [28,30]. Moreover, the induction of ICAM-1 is indispensable for thermal stimulation of lymphocyte trafficking in HEVs of lymphoid organs. These findings are in line with observations in vessels of extralymphoid organs where ICAM-1 induction is considered a signature of local inflammation. Similar increases in the ability of HEVs to support lymphocyte adhesion have been noted during natural fevers induced by LPS or turpentine [21].

A key finding is that thermal induction of lymphocyte trafficking during whole body hyperthermia is a systemic response that occurs in HEVs throughout the body [21,28,30]. Tight regulation of lymphocyte trafficking to lymphoid organs is maintained in the face of systemic activity by the fact that non-specialized venules in extralymphoid organs are refractory to direct thermal modulation of trafficking molecules (*i.e.*, CCL21, ICAM-1) [21,28,30]. The exquisite specificity of the thermal response is further evidenced by intravital observations of lymphocyte-endothelial interactions in real-time [28]. These studies showed that endothelial cells lining HEVs are highly thermally-sensitive whereas non-specialized endothelium in proximal vascular segments in the same venular tree remain unaffected. The restricted responses of HEVs to thermal stress would be predicted to provide widespread protection by focusing trafficking of naïve and central memory lymphocytes to immune-relevant sites during the early phase of the adaptive immune response.

Cooking with gas: role of IL-6 in improved immune surveillance

One of the most surprising findings is that a common cytokine effector molecule regulates thermal responses in both lymphocytes and endothelium. Even though inflammatory cytokines such as tumor necrosis factor (TNF), IL-1 β , interferon- γ (IFN- γ), and IL-6 are all potent stimulators of inflammation and endothelial expression of ICAM-1, only IL-6 was revealed to play a non-redundant role in controlling both L-selectin avidity/affinity and ICAM-1 expression [24,28,48–50]. The induction of CCL21 on HEVs by thermal stress is not mediated by IL-6 [28] and the underlying mechanism is currently under investigation.

Thermal regulation of lymphocyte-endothelial adhesion is mediated by a tightly regulated mechanism termed trans-signaling that is dependent not only on IL-6, but also an agonistic, soluble form of the IL-6 receptor (sIL-6R) binding subunit [24,28,48,50–52]. Together, these molecules act as a heterodimeric cytokine and engage the gp130 signal transduction receptor subunit. Since both IL-6 and sIL-6R must be provided at the same time and place, the dependence on IL-6 trans-signaling ensures that the thermal response is fine-tuned both temporally and spatially. This mechanism allows for targeting of specific cell types while avoiding diverse pathologic effects associated with dysregulated IL-6 signaling [48,51,53, 54].

IL-6 trans-signaling stimulates both L-selectin adhesion in lymphocytes and enhances ICAM-1 expression in HEVs in response to fever-range thermal stress [24,28,48,50]. This trans-signaling mechanism mediates thermal induction of improved L-selectin dependent adhesion in leukocytes of species representing four taxa of jawed vertebrates (mammals, avians, amphibians, teleosts) [24,26,50]. The evolutionary conservation of a cytokine-dependent, thermally-regulated adhesion response in species that evolved from a node of common ancestry ~450 million years ago suggests that this process has a strong survival advantage. Thermal stress in lymphocytes activates both the MEK1/ERK1-2 and STAT3 signal transduction pathways downstream of IL-6/sIL-6R engagement of the gp130 receptor subunit [24]. While MEK1/ERK1-2 activation is essential in controlling L-selectin association with the structural cytoskeletal matrix and consequent increases in L-selectin binding activity, the role of STAT3 in this response remains to be determined [24]. Trans-signaling is essential for IL-6 responses in endothelial cells which express limited membrane-bound IL-6R and are thus, largely unresponsive to IL-6 in the absence of sIL-6R [48,51,52,55]. The intracellular signaling pathways that regulate the response of high endothelial cells to fever-range thermal stress in vivo are currently under investigation. However, evidence that STAT3 controls ICAM-1 expression in endothelial cells in vitro [56,57] raises the possibility that this signaling pathway plays a similar role during thermally-regulated IL-6 trans-signaling in HEVs.

Collectively, studies investigating the thermal element of fever have identified a novel lymphocyte–HEV–IL-6 axis that is responsible for improving immune surveillance in secondary lymphoid organs. These observations provide support for the emerging view that IL-6 trans-signaling acts as a temporal switch regulating differential leukocyte infiltration during acute and chronic inflammation [48,51,53].

Return to baseline

Lymphocyte trafficking is by necessity a tightly regulated process. It must be swiftly induced to counter disease and infection but must also be rapidly cleared when the threat is past. Inflammation and fever are associated with high metabolic costs and chronic inflammation increases the risk of inappropriate immune interactions which can lead to autoimmune disease. Resolution, therefore, is an essential phase of an acute immune response. Noteworthy in this regard, is that a hallmark of thermal regulation of lymphocyte-endothelial adhesion in HEVs is the transient nature of the response [21,23,30]. Basal levels of lymphocyte trafficking are

restored rapidly following cessation of fever-range thermal stress. This is accomplished by a rapid return to status-quo of both L-selectin binding activity in lymphocytes and ICAM-1 density in HEVs [23,30]. Restoration of homeostasis in HEVs is achieved through active cleavage of ICAM-1 from the endothelial cell surface by a zinc-dependent metalloproteinase [30]. Zinc-dependent sheddases such as tumor necrosis factor α -converting enzyme TACE/ADAM17 and MT1-MMP have been implicated in the cleavage of endothelial ICAM-1 in endothelial cells in vitro, [58,59] suggesting that one or both of these proteases may be responsible for returning heat-induced ICAM-1 to resting levels in HEVs [30]. In addition to reducing the amount of substrate available on HEVs, sheddases could theoretically dampen lymphocyte-endothelial adhesion by increasing the local concentration of sICAM-1 which could act as a competitive inhibitor. The mechanisms leading to normalization of L-selectin binding activity are unknown but do not appear to involve zinc-dependent sheddases since no change in L-selectin expression is detected on lymphocytes during or after thermal stress [22–24]. The biphasic upregulation and attendant reduction of immune surveillance in response to fever-range thermal stress mirror the initiation and resolution steps of a well-regulated acute immune response.

Perspectives and burning questions

Inflammation is a highly regulated mediator of efficient immune surveillance, directing the trafficking of lymphocytes to induce an effective immune response. Studies of the thermal element of fever provide insight into the dynamic nature and complexity of this tightly controlled response. Fever-range thermal stress acts through a lymphocyte–endothelial–IL-6 axis as a systemic alarm system to increase the trafficking of naïve and central memory lymphocytes across HEVs in lymphoid organs throughout the body (Fig. 4). These mechanisms likely complement inflammatory cues that act locally to improve the delivery of immune cells into inflamed tissues and draining lymph nodes to fight infection and disease. Febrile temperatures fine-tune immune surveillance by regulating the function and/or expression of adhesion molecules and chemokines that participate at discrete steps in the adhesion cascade that is required for lymphocyte extravasation in HEVs. Equally important are observations that lymphocyte trafficking is rapidly restored to homeostatic levels upon cessation of thermal stress (Fig. 4). Active pruning of trafficking molecules (ICAM-1) from the vascular landscape allows the immune system to return to homeostasis, thereby avoiding pathological outcomes that result from chronic inflammation.

Major questions remain regarding the molecular mechanisms that orchestrate the rapid changes in lymphocyte trafficking across HEVs in response to the thermal element of fever. Further studies are required to dissect the relative contributions of STAT3 versus MEK1/ERK1-2 signaling pathways for improved L-selectin adhesion and ICAM-1 induction. It is not known what specific zinc-dependent ectoenzymes are responsible for shedding of ICAM-1 from HEVs following cessation of thermal stress. It also remains to be determined if ICAM-1 cleavage from high endothelial cells following thermal stress is mediated by endothelial cells, or if zinc-dependent sheddases on adherent leukocytes act in *trans* to release ICAM-1 from HEVs. An intriguing line of investigation will be to determine if the activity of these proteases is regulated by thermal stress or IL-6. Another outstanding question relates to what cells provide the IL-6 and sIL-6R that drive the thermal stress response. Many cell types are a source of IL-6 (*e.g.*, endothelial cells, fibroblasts and leukocytes) whereas production of sIL-6R is restricted to hepatocytes and some leukocytes. Finally, it remains to be examined if the molecular basis underlying the site-restricted activity of fever-range thermal stress depends on properties intrinsic to the high endothelial cells that line HEVs, or if influences of the surrounding cytokine-rich microenvironment of lymphoid organs are predominant.

The picture to emerge from these studies is that the molecular mechanisms that regulate lymphocyte trafficking in HEVs during the initiation and resolution of acute inflammatory responses are highly integrated and subject to multiple regulatory checks and balances. Lessons learned from the dynamic nature of lymphocyte trafficking across HEVs are likely to provide fertile ground for the development of novel strategies to control immune surveillance under conditions where it would be beneficial to either enhance the immune response (*e.g.*, to fight infection or cancer development) or to temper immunity (during chronic inflammation or in autoimmune disorders).

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Figure 1.

The schematic depicts the biphasic nature of the inflammatory response triggered by inflammatory stimuli that accompany infection such as systemic fever. The initiation phase of acute inflammation is characterized by the rapid transition from steady-state homeostatic levels of lymphocyte trafficking across vascular endothelial barriers to elevated, peak levels of extravasation. Resolution of inflammation is dependent on the rapid restoration of lymphocyte trafficking to normal steady-state levels.

Figure 2.

Fever-range thermal stress targets discrete molecular events in the multistep adhesion cascade that governs lymphocyte trafficking across high endothelial venules (HEVs). Molecules regulated by fever-range temperatures are indicated by red text in the schematic. Photomicrograph shown in lower panel depicts a typical PNAd⁺ HEV (indicated by green immunofluorescence stain) in a murine lymph node cryosections. Bar = 100 μ m. Note the cuboidal morphology of the endothelial cells lining the HEV. The white dotted line demarks the boundary of the HEV.

Figure 3.

Fever-range thermal stress dynamically regulates lymphocyte trafficking across HEVs of peripheral lymph nodes. *Left panel* depicts the homing of fluorescent-labeled (TRITC, red) naïve lymphocytes across cuboidal high endothelial venules (HEVs; demarked by white dotted line) was investigated in short-term (1 h) homing assays after intravenous injection of labeled lymphocytes. Lymph node cryosections were subsequently counterstained for the expression of ICAM-1 (green). Photomicrographs show the distribution of TRITC-labeled lymphocytes in ICAM-1⁺ HEVs in peripheral lymph nodes of mice treated with fever-range thermal stress (FRTS) or normothermal controls (NT). Bar = 10 μ m. Note the relative increase in TRITC-labeled lymphocytes and ICAM-1 staining intensity in HEVs following thermal stress. *Middle panels*, histograms depict the immunofluorescence intensity of ICAM-1 staining quantified in all HEVs in a cross-section of lymph nodes by computer-assisted image analysis; the mean fluorescence intensity (MFI) is indicated for each condition. *Right panels* indicate the number of TRITC-labeled cells in lymph node cryosections detected by fluorescence microscopy (n = 10 fields). * $p < 0.007$ by Student's *t*-test for difference between normothermal and fever-range thermal stress conditions.

Figure 4.

Thermal enhancement of immune surveillance depends on increased expression of trafficking molecules such as ICAM-1 on HEVs. This initial phase is dependent on IL-6. Following cessation of thermal stress, ICAM-1 expression and lymphocyte trafficking are restored to homeostatic levels by zinc-dependent sheddase activity. Membrane and soluble (s) forms of ICAM-1 are depicted.