Fever and the thermal regulation of immunity: the immune system feels the heat

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Abstract | Fever is a cardinal response to infection that has been conserved in warm-blooded and cold-blooded vertebrates for more than 600 million years of evolution. The fever response is executed by integrated physiological and neuronal circuitry and confers a survival benefit during infection. In this Review, we discuss our current understanding of how the inflammatory cues delivered by the thermal element of fever stimulate innate and adaptive immune responses. We further highlight the unexpected multiplicity of roles of the pyrogenic cytokine interleukin-6 (IL-6), both during fever induction and during the mobilization of lymphocytes to the lymphoid organs that are the staging ground for immune defence. We also discuss the emerging evidence suggesting that the adrenergic signalling pathways associated with thermogenesis shape immune cell function.

The fever response is a hallmark of infection and inflammatory disease, and has been shaped through hundreds of millions of years of natural selection. Febrile temperatures are so closely linked to the inflammatory response that heat (*calor*) is one of the four cardinal signs of inflammation, along with pain (*dolor*), redness (*rubor*) and swelling (*tumor*), as described by Celsus in approximately the first century BC[1]. The induction of fever in endothermic (warm-blooded) animals occurs at a high metabolic cost, such that a 1 °C rise in body temperature requires a 10–12.5% increase in metabolic rate[2]. There is mounting evidence that the increase in core body temperature of 1 °C to 4 °C that occurs during fever is associated with improved survival and the resolution of many infections. For example, the use of antipyretic drugs to diminish fever correlates with a 5% increase in mortality in human populations infected with influenza virus and negatively affects patient outcome in the intensive care unit of hospitals[3–5]. Preclinical studies in rabbits infected with rinderpest virus also found an increase in mortality when fever was inhibited using the antipyretic drug acetylsalicylic acid (also known as aspirin): 70% of acetylsalicylic acid-treated animals died as a result of infection compared with only 16% of the animals that had a normal febrile response[6]. However, fever is not universally beneficial, particularly in cases of extreme inflammation where lowering rather than raising body temperature has evolved as a protective mechanism[7–10]. Thus, uncontrolled fever is associated with worse outcomes in patients with sepsis or neurological injuries, whereas treatments that induce hypothermia can have a clinical benefit in these patients[11,12]. A challenge in ascertaining the precise value of fever in endotherms is that the antipyretics used to inhibit fever target multiple aspects of the inflammatory response in addition to temperature regulation[11].

Ectothermic (cold-blooded) vertebrates, which last shared a common ancestor with mammals more than 600 million years ago, provide an 'experiment in nature' by which to examine the direct impact of febrile temperatures on survival. Ectotherms as diverse as reptiles, fish and insects raise their core temperature during infection through behavioural regulation, which leads the animals to seek warmer environments (despite the risk of predation) or, in the case of bees, to raise the local temperature of the hive through increased physical activity[2,13–19]. Landmark studies published 40 years ago by Kluger's laboratory showed that the survival of the desert iguana *Dipsosaurus dorsalis* is reduced by 75% if prevented from behaviourally raising its core temperature by approximately 2 °C after infection with the Gram-negative bacterium *Aeromonas hydrophila*[2,13,14]. The heat-seeking behaviour of the desert iguana, blue-finned tuna and leech is negated by antipyretic drugs, indicating that common biochemical pathways drive fevers in ectothermic and endothermic animals[14,16,20]. Surprisingly, the correlation between infection

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and increased temperature even extends to plants, which arose 1.5 billion years ago. For example, the temperature of the leaves from the bean plant *Phaseolus vulgaris* increases by around 2 °C following infection with the fungus *Colletotrichum lindemuthianum*[21]. Thermoregulation in plants occurs through mitochondrial respiration[22], although it is not known whether these fever-like responses have a direct effect on the clearance of infection.

The fact that fever has been retained throughout vertebrate evolution strongly argues that febrile temperatures confer a survival advantage. A long-standing mystery relates to the protective mechanisms by which fever wards off attacks by invading pathogens. One mechanism involves direct effects of febrile temperatures on the infectious potential of pathogens[23]. For example, 40–41 °C temperatures cause a greater than 200-fold reduction in the replication rate of poliovirus in mammalian cells and increase the susceptibility of Gram-negative bacteria to serum-induced lysis[24,25]. In this Review, we discuss the evidence suggesting that febrile temperatures boost the effectiveness of the immune response during infections by stimulating both the innate and the adaptive arms of the immune system. We highlight the role of the pyrogenic cytokine interleukin-6 (IL-6) in two key phases of the febrile response: first, in driving the rise in core temperature; and second, as a downstream effector cytokine that orchestrates lymphocyte trafficking to lymphoid organs. We also describe febrile temperature as a 'rheostat' that 'dials down' systemic inflammation during the return to homeostasis. In addition, we highlight new data demonstrating the overlapping signalling pathways that are involved in thermogenesis and in the regulation of the immune response. We only briefly discuss the neuronal circuitry that drives fever and the evolutionarily conserved heat shock protein (HSP) response (BOX 1), but we refer the reader to recent comprehensive reviews for additional information on these topics, as well as on the contributions of hypothermia to limiting inflammation[26–30].

Box 1 | **Thermal regulation of heat shock proteins**

Heat shock proteins (HSPs) are cytoprotective proteins that are constitutively expressed and also rapidly induced under proteotoxic stress conditions such as heat, hypoxia, oxidative stress, toxin exposure, nutrient deprivation and infection[26–29,200–202]. Although HSPs were originally discovered in the context of heat shock (42–45 °C), they are also inducible by febrile temperatures (38–41 °C) in mammalian cells[26,122,189–191]. Stress-induced transcription of HSPs is driven by post-translational modifications (sumoylation and phosphorylation) of heat shock factor protein 1 (HSF1): these modifications release HSF1 from a complex with HSP70 and HSP90 (REFS 29,200,201). This results in the formation of HSF1 homotrimers that translocate to the nucleus and activate the transcription of genes including those encoding HSPs that contain heat shock element sequences[29,200,201]. A major function of HSPs is to maintain appropriate folding of their client proteins, thereby protecting them from proteolysis. HSPs have key roles in regulating multiple signalling pathways under constitutive and stress conditions. For example, there are more than 200 established client proteins of HSP90, including members of the mitogen-activated protein kinase (MAPK), Janus kinase–signal transducer and activator of transcription (JAK–STAT) and cyclin-dependent kinase 1 (CDK1) signalling pathways[29,200,202–204]. Cancer cells that are under chronic proteotoxic stress conditions often become 'addicted' to HSPs, and high intratumoural expression of HSP70 or HSP90 is a poor prognostic indicator in patients with cancer, suggesting HSP inhibitors as a treatment option in cancer[200,203,204]. There are also non-canonical HSPs that do not have traditional chaperone or protein-folding activity but their expression is nonetheless tightly regulated by HSF1. One example is CXC-chemokine ligand 8 (CXCL8; also known as IL-8), which mediates the recruitment of neutrophils upon exposure to fever-range hyperthermia in lipopolysaccharide instillation models of acute lung inflammation[84,85]. Active areas of investigation in the HSP field are considering the physiological impact of the multiple post-translational modifications of HSFs and HSPs (for example, phosphorylation, acetylation, *S*-nitrosylation, ubiquitylation and sumoylation), as well as the interplay between these molecules and positive and negative immune regulation[29,200,205].

Induction of fever

The IL-6–COX2–PGE2 axis drives fever. The induction and maintenance of fever during infection involves the tightly coordinated interplay between the innate immune system and the neuronal circuitry within the central and peripheral nervous systems. Immune sensing of infection begins with the binding of pathogen-associated molecular patterns (PAMPs) — for example, lipopolysaccharide (LPS), viral RNA and fungal sugars — to pathogen recognition receptors (PRRs), such as Toll-like receptors (TLRs), which are expressed by innate immune cell populations, including macrophages, neutrophils and dendritic cells (DCs) (FIG. 1). Much of our current understanding of the molecular mechanisms underlying fever stems from studies in which rodents were injected with LPS, which is a component of Gram-negative bacterial cell walls, to model immune-induced thermoregulation. In this model, prostaglandin E2 (PGE2) produced by brain vascular endothelial cells is considered to be a major pyrogenic mediator of fever[31–33]. This lipid effector molecule integrates input signals from pyrogenic cytokines that are produced in response to pathogenic stimuli with output signals involving neurotransmitters that raise core body temperature (FIG. 1). PGE2 is also synthesized in the periphery early in this response — that is, before the detection of circulating cytokines. It is produced by haematopoietic cells following LPS-mediated activation of TLR4 and travels through the blood–brain barrier to initiate fever[26,30,34–38]. LPS-induced fever occurs via autonomic mechanisms driven by PGE2 binding to PGE2 receptor 3 (EP3; also known as PTGER3), which is expressed by thermoregulatory neurons in the median preoptic nucleus region of the hypothalamus[8,39–41]. Endotherms elevate body temperature through the release of noradrenaline, which increases thermogenesis in brown adipose tissue and induces vasoconstriction in the extremities to reduce passive heat loss[26,27]. In addition, signalling through the neurotransmitter acetylcholine stimulates the musculature to convert stored chemical energy into thermal energy and increases overall metabolic rates[2,26,42,43]. Endotherms, like ectotherms, also engage in heat-seeking behavioural thermoregulation that does not require median preoptic neurons, although the pathways involved are mostly unknown[8–10].

LPS-induced TLR4 signalling stimulates the synthesis of pyrogenic cytokines (namely, IL-1, IL-6 and tumour necrosis factor (TNF)) at the site of infection, as well as in the brain, and it is becoming clear that IL-6 is an important mediator of fever induction[26,44–47]. Notably, multiple cell types in the brain (for example, astrocytes, microglial cells and neurons) have the ability to synthesize IL-6 in response to local inflammatory stimuli[48–53]. Although the direct administration of IL-1, IL-6 or TNF into the brain leads to a febrile response, several lines of evidence

point to a requisite role for IL-6 in sustaining fever. In this regard, LPS-induced fever does not occur in the presence of IL-6-specific neutralizing antibody or in IL-6-deficient mice, even though TNF and IL-1 upregulation is normal in these settings[54–58]. Moreover, direct intracerebroventricular injection of IL-6, but not IL-1, restores febrile responses in IL-6-deficient mice[55]. Febrile temperatures have further been implicated in a positive feedback loop during the early stages of infection. Specifically, passive elevation of the core body temperature of mice to the febrile range using whole-body hyperthermia substantially augments circulating levels of IL-1, IL-6 and TNF during LPS-induced inflammation[26,59–61]. The pyrogenic role of IL-6 has recently been corroborated in patients with paediatric leukaemia, in which treatment with the IL-6 receptor antagonist tocilizumab was found to reverse the high fevers that develop during T cell based-immunotherapy (specifically, following the administration of chimeric antigen receptor-expressing T cells or a CD19/CD3-bispecific antibody)[62,63].

Systemic or locally produced cytokines act in the brain to augment the synthesis of cyclooxygenase 2 (COX2), the enzyme responsible for oxidizing arachidonic acid to produce PGE2 (FIG. 1), and IL-1 receptors that mediate COX2 induction have been identified on brain endothelial cells within the median preoptic nucleus region of the hypothalamus[64,65]. Although the specific cell types that upregulate COX2 expression in response to IL-6 remain to be identified, blood vessels in the brain reportedly express the IL-6 receptor subunit-α (IL-6Rα)[53], which together with the ubiquitously expressed gp130 subunit (also known as IL-6Rβ) forms the functional IL-6 receptor. Several studies have shown that cerebral COX2, PGE2 and fever are not induced during LPS-driven inflammation in IL-6-deficient mice or in the presence of IL-6-specific neutralizing antibody[66–68]. Alternatively, IL-6 cannot initiate a febrile response in the absence of COX2 or PGE2, and intracerebroventricular delivery of PGE2 bypasses the requirement for IL-6 during fever induction in

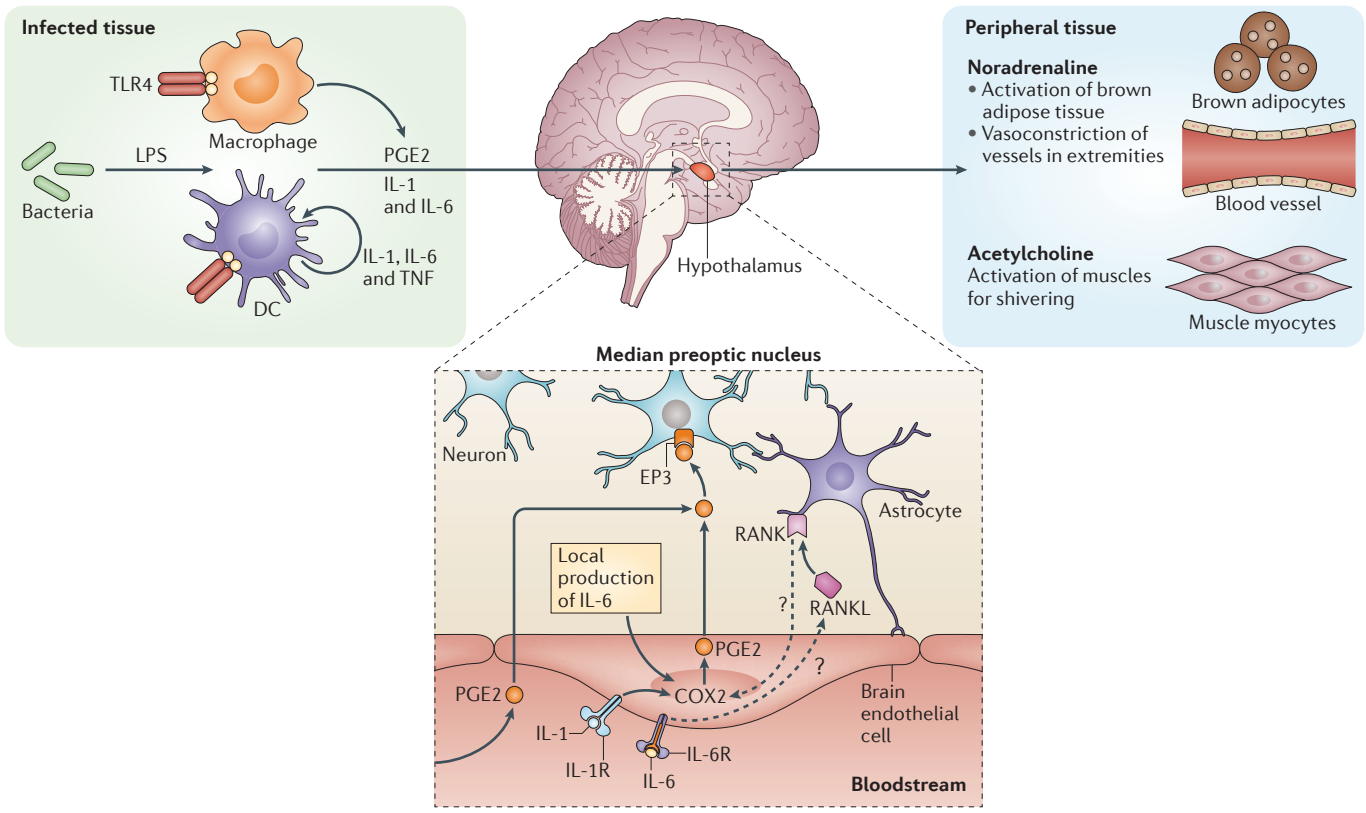


Figure 1 | **The induction of fever during infection.** The recognition of damage-associated molecular patterns (DAMPs) or pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharide (LPS), by Toll-like receptors (TLRs) and other pattern recognition receptors drives the activation of dendritic cells (DCs) and macrophages (upper left panel). These innate immune cells release prostaglandin E2 (PGE2) and pyrogenic cytokines (namely, interleukin-1 (IL-1), IL-6 and tumour necrosis factor (TNF)) that act systemically to induce fever. IL-6 operates downstream of IL-1 in the median preoptic nucleus region of the hypothalamus to induce the synthesis of cyclooxygenase 2 (COX2), the enzyme responsible for the production of additional PGE2 (REFS 64,65). PGE2 is considered to be the major pyrogenic mediator of fever[31–33]. Receptor activator of NF-κB (RANK) that is expressed by astrocytes also acts via the COX2–PGE2 pathway to induce fever[47]. However, it is not known whether this pathway parallels the IL-6 response or whether the IL-6 and RANK ligand (RANKL) pathways converge, potentially via IL-6 regulation of RANKL expression in vascular endothelial cells in the hypothalamus. Neurons expressing PGE2 receptor 3 (EP3) trigger the sympathetic nervous system to release noradrenaline, which elevates body temperature by increasing thermogenesis in brown adipose tissue and by inducing vasoconstriction to prevent passive heat loss (upper right panel)[2,26,27,42,43]. In addition, acetylcholine contributes to fever by stimulating muscle myocytes to induce shivering. IL-1R, IL-1 receptor; IL-6Rα, IL-6 receptor subunit-α.

IL-6-deficient mice[69,70]. Collectively, these observations establish that COX2 and PGE2 are crucial mediators that can operate downstream of IL-6 in the LPS-induced febrile response.

RANKL and fever induction. An open question is whether IL-6 is the direct regulator of COX2 and PGE2 induction during the febrile response or whether other intervening cytokines are involved. The possibility of other cytokine involvement is suggested by an elegant study by Hanada *et al.*[47] who showed that, similarly to IL-6, the cytokine receptor-activator of NF-κB ligand (RANKL; also known as TNFSF11) converges on the COX2–EP3–PGE2 pathway, leading to fever induction in the LPS-induced model of inflammation (FIG. 1). RANKL is best known as a regulator of bone remodelling and lymph node organogenesis[71]. However, mRNA encoding RANKL is also produced in the lateral septal nucleus region of the brain that interconnects with the hypothalamus, and the RANKL receptor, RANK (also known as TNFRSF11A), is found on astrocytes in the preoptic region of the hypothalamus[47]. Further support for a role of this cytokine in thermoregulation is provided by findings that children with *RANK* mutations exhibit impaired fever responses during pneumonia[47]. Although the potential interplay between IL-6 and RANKL–RANK during fever has not been explored, it is tempting to speculate that RANKL is a downstream mediator of IL-6-induced pyrogenesis on the basis of evidence that IL-6 directly stimulates RANKL synthesis by synovial fibroblasts in mouse models of rheumatoid arthritis[72].

Immune stimulation by thermal stress

One benefit widely attributed to fever is the enhancement of immune-protective mechanisms during infection. Defence against pathogens involves tight spatial and temporal regulation of the immune system, and the same pyrogenic cytokines that are produced during the induction of fever also operate locally to orchestrate immunity within infected tissues[73]. Innate immune cells are the 'first responders', arriving within hours of infection to directly destroy pathogens via phagocytic or cytotoxic activities. These activities limit infection until a peak adaptive immune response is generated, normally around 1 week later. Macrophages and DCs bridge the gap between innate and adaptive immunity by taking up pathogens in peripheral tissues and then relocating to draining lymph nodes where they drive the population expansion of pathogen-specific effector T cells[74,75]. Crucial to this process is the colocalization of DCs and T cells near high endothelial venules (HEVs), which are the major portals for entry of blood-borne lymphocytes[74–76].

Given the complexity of these immune mechanisms, it is remarkable that fever-range temperatures stimulate almost every step of this process, promoting both innate and adaptive immunity. In the various *in vitro* and *in vivo* studies described below, the potential impact of the thermal element of fever has primarily been explored using hyperthermic temperatures within the febrile range for mammals (that is, ranging from 38 °C to 41 °C; ΔT ~1–4 °C above baseline). Experimental hyperthermia is a powerful approach to study the impact of fever-range temperatures on immunity, which is otherwise difficult to discriminate during natural fever because of the attendant inflammatory programme (comprised of lipid and cytokine mediators) that regulate both fever and immunity. However, an important caveat from a physiological perspective is that the heat conservation associated with natural fever fundamentally differs from the cooling mechanisms that are enacted by thermoregulation following exogenous heat application.

Impact of febrile temperatures on innate immunity. Previous research using animal models of hyperthermia treatment alone, or with LPS challenge or bacterial infection, strongly supports the idea that fever-range temperatures elevate the respiratory burst that is typically associated with the activation and bacteriolytic activity of neutrophils[77,78] (FIG. 2a). Thermal stress further increases neutrophil recruitment to local sites of infection and other distant tissues[61,79] (FIG. 2a), including tumours[77]. Neutrophil localization in peripheral tissues is at least partly due to heat-induced increases in the numbers of circulating neutrophils, which are dependent on granulocyte colony-stimulating factor (G-CSF)[80,81]. G-CSF is also central to a model of radiation-induced neutropenia in which fever-range whole-body hyperthermia substantially increases the number of neutrophils in the blood and augments the number of haematopoietic stem cells and neutrophil progenitors in the bone marrow[82] (FIG. 2a). This effect is dependent on enhanced production of IL-1α, IL-1β and IL-17 preferentially in intestinal tissue. Importantly, the precise outcome of the thermal effect depends on the heating protocol used and the geography of cell recruitment (FIG. 2a). Indeed, temperatures above the normal febrile range impair neutrophil accumulation and function[83]. Moreover, Hasday and colleagues[61,84] found that fever, or exposure to fever-range hyperthermia, in an LPS model increases neutrophil localization to the lungs, which can have negative consequences owing to inflammation-induced local tissue damage. Heat-induced neutrophil recruitment in the lungs depends on the non-canonical chemotactic HSP CXC-chemokine ligand 8 (CXCL8; also known as IL-8), the expression of which is controlled by the heat-inducible transcription factor heat shock factor protein 1 (HSF1)[61,84,85] (BOX 1). Neutrophil recruitment to the lungs also involves a decrease in endothelial barrier integrity through a mechanism that depends on the mitogen-activated protein kinases (MAPKs) p38, extracellular signal-regulated kinase 1 (ERK1) and ERK2 (REF. 84).

The effect of heat on natural killer (NK) cells has been most extensively studied in the context of tumour immunity. It has been shown that NK cell cytotoxic activity and recruitment to tumour sites is increased by fever-range hyperthermia *in vivo*[86–89] (FIG. 2b). This enhanced cytotoxicity depends on heat-induced upregulation of the NKG2D ligand MICA (MHC class I polypeptide-related sequence A) on tumour cells, as well as on the clustering of NKG2D receptors on the surface of NK cells[90]. Elevated temperatures also decrease MHC class I expression by tumour cells while simultaneously

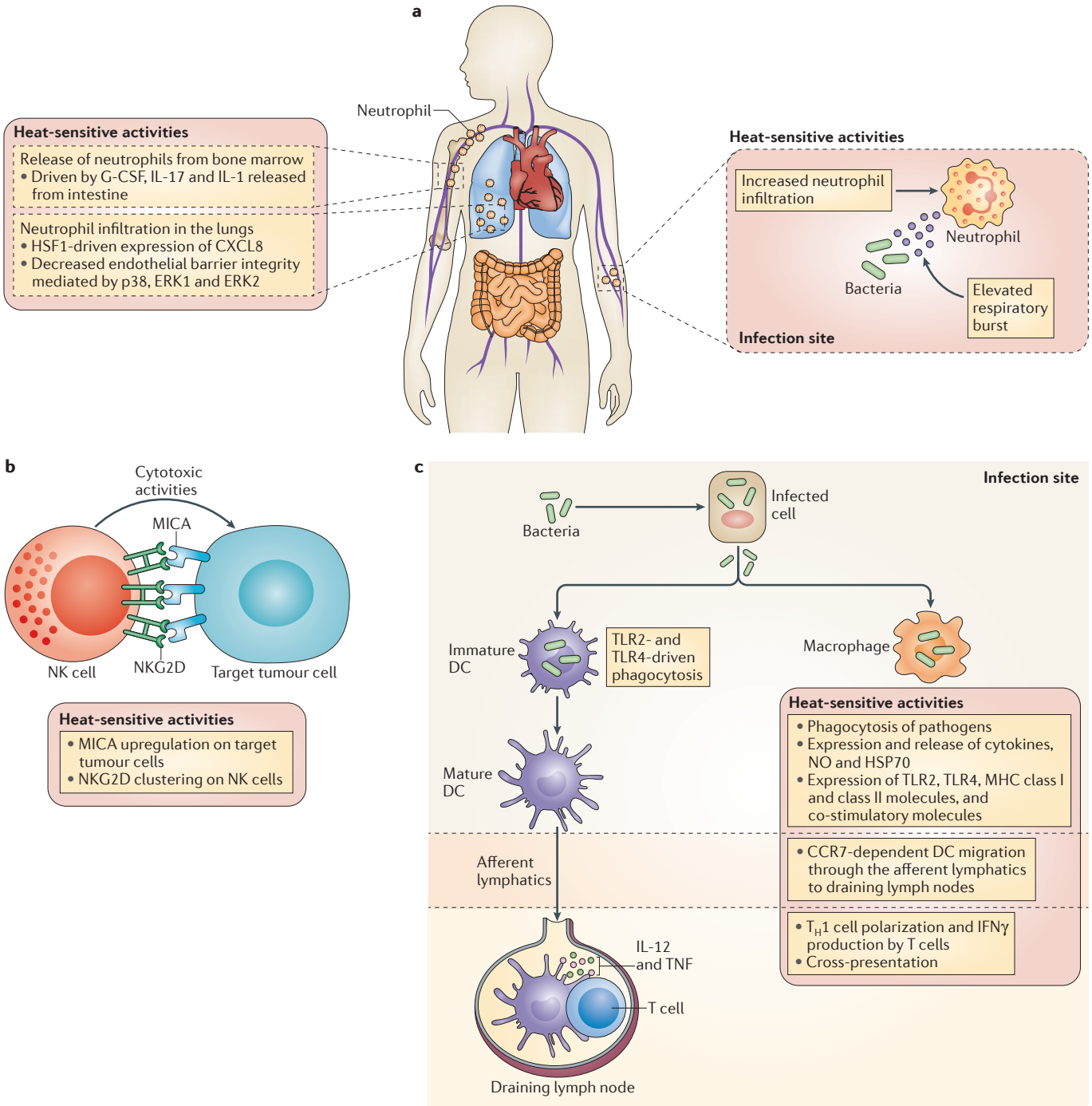


Figure 2 | **Response of innate immune cells to thermal stress.** **a** | Fever-range temperatures drive several crucial aspects of innate immunity. Fever-range hyperthermia stimulates the release of neutrophils from the bone marrow in a granulocyte colony-stimulating factor (G-CSF)-driven manner (left panel)[80–82]. Febrile-range temperatures also promote neutrophil recruitment to the lungs and other local sites of infection in a CXC-chemokine ligand 8 (CXCL8)-dependent manner that additionally involves decreased endothelial barrier function in blood vessels[61,84,85]. Upon arriving at the site of infection, thermal stress further elevates the respiratory burst, which increases the bacteriolytic activity of neutrophils (right panel)[77,78]. **b** | Thermal treatment improves natural killer (NK) cell cytotoxic activity through the induction of MHC class I polypeptide-related sequence A (MICA) expression on target cells (for example, tumour cells) and by inducing the clustering of the MICA receptor NKG2D on the surface of NK cells[90]. **c** | Temperatures in the febrile range increase the ability of antigen-presenting cells to support the formation of the adaptive immune response. Heat improves the phagocytic potential of macrophages and dendritic cells (DCs) and increases their responsiveness to invading pathogens by upregulating their expression of both Toll-like receptor 2 (TLR2) and TLR4 (REFS 119,120). Thermal treatment also induces the release of immunomodulatory molecules such as cytokines (for example, tumour necrosis factor (TNF)), nitric oxide (NO) and heat shock protein 70 (HSP70). In addition, heat increases the expression of MHC class I and MHC class II molecules, as well as co-stimulatory molecules (CD80 and CD86), by mature DCs and augments their CC-chemokine receptor 7 (CCR7)-dependent migration via the afferent lymphatics that serve as a conduit to draining lymph nodes[117,121–124]. DCs exposed to febrile temperatures are also more efficient at cross-presenting antigens and inducing T helper 1 (T_H1) cell polarization[121]. ERK, extracellular signal-regulated kinase; HSF1, heat shock factor protein 1; IFNγ, interferon-γ; IL, interleukin.

increasing HSP70 production, and both of these responses are linked to enhanced cytotoxic potential in NK cells[91]. The upregulation of HSPs in tumour cells in response to thermal stress is also likely to be involved in the enhanced cross-priming of antigen-specific cytotoxic T lymphocytes that was observed when DCs were loaded with lysate from heated melanoma cells[92].

Macrophages have served as a major model for the study of fever-range hyperthermia. Early studies demonstrated that whole-body heating (to ~39.5 °C) improves bacterial clearance and also increases serum concentrations of IL-1, IL-6 and TNF in mice challenged with LPS[59,60,93,94]. The source of these cytokines was found to be the macrophages of the liver (that is, Kupffer cells), as well as macrophages in other organs. Later work by Lee *et al.*[95] showed that hyperthermia induces the upregulation of HSP70 and that this 'reprogrammes' macrophages to show sustained activation in response to LPS. The mechanism involves the phosphorylation of inhibitor of NF-κB (IκB) and IκB kinase (IKK), the nuclear translocation of nuclear factor-κB (NF-κB) and its binding to the *Tnf* promoter[95,96]. HSP70 is also required for enhancing the expression of nitric oxide and inducible nitric oxide synthase by peritoneal macrophages following exposure to fever-range temperatures together with LPS and interferon-γ (IFNγ)[97]. Although HSPs are usually assumed to be intracellular, heat stress can induce the release of HSP70 from cells into the extracellular environment where it can act as a damage-associated molecular pattern (DAMP) to stimulate macrophages and DCs[98–100]. Extracellular HSP70 and other HSPs engage multiple surface receptors, including CD91 (also known as LRP1), scavenger receptor A, CD40, TLR2 and TLR4, leading to the release of nitric oxide, TNF, IL-1β, IL-6 and IL-12 (REFS 100–110). Notably, some investigators have paradoxically observed an anti-inflammatory role for HSPs[111–113]. It has been suggested that these differences result from the precise location of the HSPs within macrophages: extracellular HSPs provide danger signals to enhance inflammation, whereas intracellular HSPs could help to suppress inflammatory signalling[114]. Taken together, the data regarding innate immune cells, body temperature and HSPs reveal fascinating, but still poorly understood, layers of interdependency between the febrile response and the more ancient HSP response.

Fever enhances DC functions. Several studies have demonstrated that elevated temperatures substantially enhance the phagocytic potential of DCs, in addition to augmenting IFNα production in response to viral infection[115–118] (FIG. 2c). Heating of immature DCs also upregulates their expression of TLR2 and TLR4, suggesting a role for thermal signals in enhancing pathogen sensing by innate immune cells[119,120]. Febrile temperatures further increase DC expression of MHC class I and MHC class II molecules and co-stimulatory molecules, including CD80 and CD86, and can augment the secretion of the T helper 1 (T_H1) cell-polarizing cytokines IL-12 and TNF[102,117,119–123]. Additional reports point to a role for fever-range temperatures in augmenting the migration of antigen-presenting cells (APCs), such as skin Langerhans cells, to draining lymph nodes[124] (FIG. 2c). These data may help to explain the fact that febrile temperatures can accelerate the swelling phase of a contact hypersensitivity reaction when heat is delivered to mice shortly after the application of the elicitation dose of a skin sensitizer, fluorescein isothiocyanate (FITC)[124]. The underlying mechanism that directs DC migration to draining lymph nodes probably involves increased responsiveness of CC-chemokine receptor 7 (CCR7) to its ligands, which has been described for heat-treated mature DCs in chemotaxis assays *in vitro*[121]. CCR7 senses CC-chemokine ligand 21 (CCL21) gradients *in vivo*, thereby guiding DC entry into afferent lymphatics and their subsequent migration near HEVs within draining lymph nodes[125–128]. Thus, febrile temperatures seem to regulate the CCR7–CCL21 axis in order to optimally position DCs in lymphoid organs at sites where they can present antigen to lymphocytes upon their arrival via HEVs.

Given these observations, it is not surprising that fever-range thermal stress enhances the ability of DCs to stimulate T cells, as well as DC cross-presenting functions (FIG. 2c). In mixed lymphocyte reactions, applying thermal stress *ex vivo* to LPS-pulsed mature human monocyte-derived DCs led to enhanced proliferation of naive $CD4^+$ T cells and promoted their differentiation towards a T_H1 cell phenotype[121]. Similarly, DCs isolated from heat-exposed mice exhibit a superior ability to activate T cells[102]. In studies in which DCs from patients with medullary thyroid cancer were pre-heated before co-culture with T cells, the T cells showed enhanced cytotoxicity against tumour targets[119]. This increased cytotoxicity of effector T cells correlated with heat-induced upregulation of both MHC class I molecule and HSP70 expression in mature, but not immature, DCs. Together, these findings demonstrate that systemic fever-range temperatures can target different components of the innate immune system, including the HSP response, in order to enhance effector T cell responses.

Thermal mechanisms boost adaptive immunity. A crucial determinant for the generation of adaptive immunity is the high rate of lymphocyte trafficking through lymphoid organs. The entire pool of naive T cells in a mouse lymph node turns over around two to three times per day as a result of T cell recirculation[75,129]. This dynamic flux increases the probability that rare antigen-specific T cells (present at a frequency of only ~1 in 10^5–10^6)[130,131] will receive activating signals from DCs. The entry of blood-borne B cells and T cells into lymph nodes and Peyer's patches occurs preferentially at HEVs through a well-defined adhesion cascade that involves several steps: first, L-selectin- and/or α4β7 integrin-initiated tethering and rolling; second, CCL21-dependent activation of CCR7 on adherent lymphocytes; third, lymphocyte function-associated antigen 1 (LFA1)-mediated firm arrest via binding to its endothelial counter-receptors intercellular adhesion molecule 1 (ICAM1) and ICAM2; and fourth, LFA1–ICAM-directed transendothelial migration[74–76,132,133]. As described below, we have shown that fever-range thermal stress targets multiple steps in this cascade by invoking a wide array of lymphocyte and endothelial trafficking molecules[134–142] (FIG. 3a).

An early indication that fever could control lymphocyte trafficking emerged from studies showing transient decreases in circulating levels of T cells in mice or patients with cancer following elevation of core body temperatures to ~39.5 °C by febrile-range whole-body hyperthermia[83,137,143]. Reductionist studies found that direct heat treatment of B cells or T cells *ex vivo* for 6 hours resulted in an approximately twofold increase in their ability to bind to HEVs *in vitro* or to home to lymph nodes or Peyer's patches *in vivo*[134–139]. Lymphocytes isolated from heat-exposed mice exhibit similar enhancement of homing properties[138]. It is worth noting that this represents a substantial increase above the already efficient rate of homeostatic trafficking whereby approximately one in four lymphocytes initiate the adhesive events that precede extravasation[75,129].

Fever-range temperatures augment trafficking through a lymphocyte-autonomous mechanism by targeting the binding activity of both L-selectin (FIG. 3a) and α4β7 integrin without altering their density[134,136–139]. In lymph node HEVs, fever-range hyperthermia promotes L-selectin-dependent lymphocyte rolling along vessel walls through the formation of short-lived catch-bonds with its endothelial counter-receptor, peripheral node addressin (PNAD)[74,75]. Febrile temperatures also enhance α4β7 integrin binding to mucosal addressin cell adhesion molecule 1 (MADCAM1) in HEVs in Peyer's patches and mesenteric lymph nodes[144]. Direct exposure of lymphocytes to heat does not alter the affinity of LFA1 for its endothelial ligands[134,136]. It remains an open question whether the chemokine receptor CCR7 is affected by febrile temperatures.

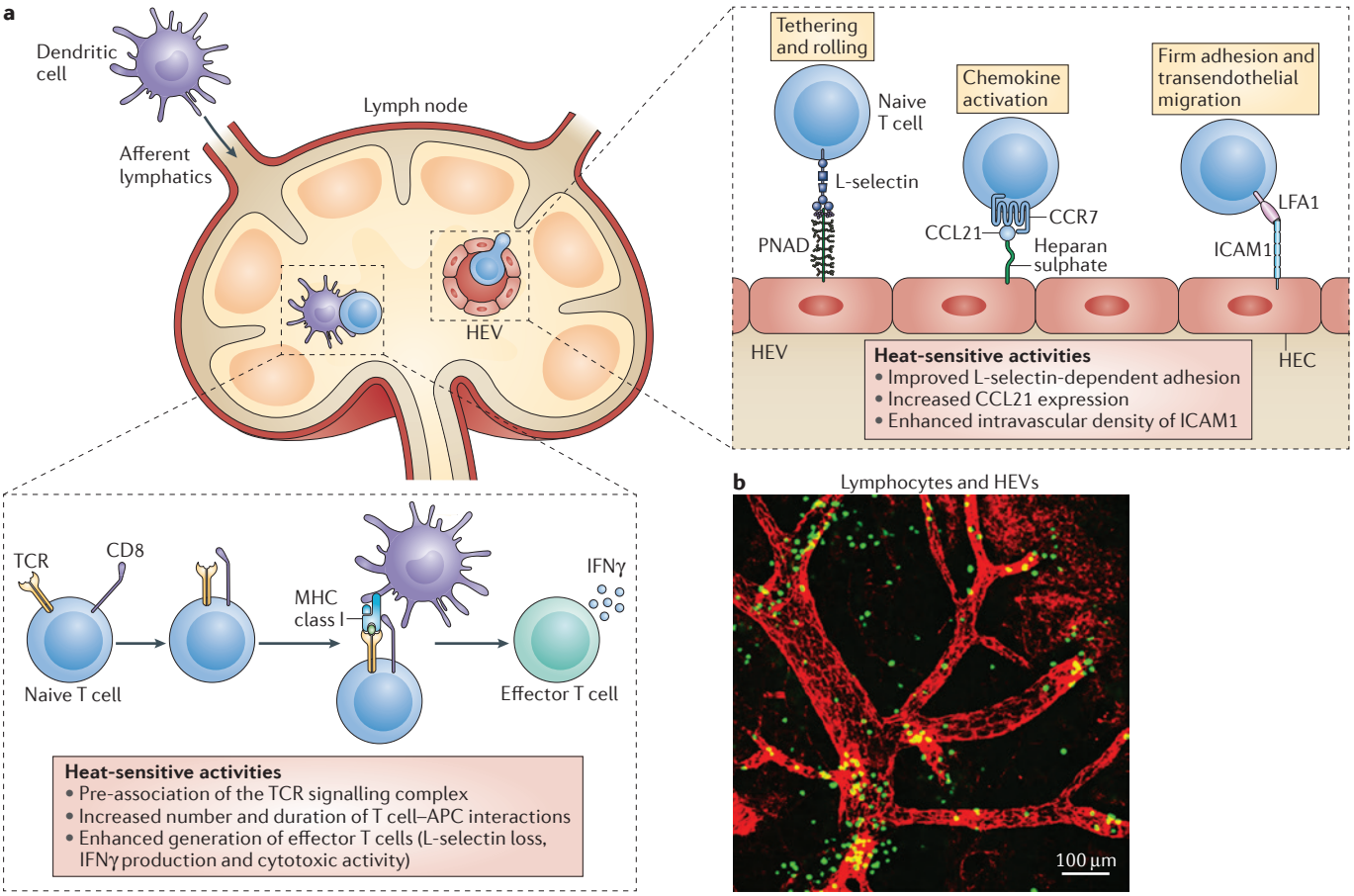


Figure 3 | **Fever-range thermal stress and the adaptive immune response. a** | Fever-range thermal stress supports increased adaptive immunity by targeting two distinct aspects of T cell activation in lymph nodes. Heat enhances the rate of lymphocyte trafficking across high endothelial venules (HEVs) in peripheral lymph nodes through effects on each step of the adhesion cascade. Heat treatment of lymphocytes increases the frequency of L-selectin-dependent tethering and rolling interactions[134,135,137–139]. Febrile-range temperatures independently act on HEVs to enhance the transition of lymphocytes from transient rolling to stable arrest by increasing the intravascular density of CC-chemokine ligand 21 (CCL21) on the heparan sulphate-rich glycocalyx and intercellular adhesion molecule 1 (ICAM1)[140–142]. ICAM1 also supports lymphocyte crawling to inter-endothelial cell junctions and transendothelial migration[131,145,146]. Heat also acts directly on the T cells within lymphoid organs by pre-clustering components of the immunological synapse (the T cell receptor (TCR) β-chain and CD8). This prolongs stable contacts with antigen-presenting cells (APCs) and increases $CD8^+$ T cell differentiation towards an effector phenotype that is characterized by enhanced L-selectin downregulation, cytotoxic function and production of interferon-γ (IFNγ)[151,152]. **b** | Epifluorescence whole-mount confocal microscopy image of HEVs that are actively supporting lymphocyte trafficking in a mouse lymph node. HEVs are stained in red with phycoerythrin (PE)-conjugated MECA-79 antibody that recognizes peripheral lymph node addressin (PNAD), whereas lymphocytes are labelled in green using carboxyfluorescein succinimidyl ester (CFSE). Photomicrograph image of lymph node HEVs courtesy of J. Muhitch, Roswell Park Cancer Institute, Buffalo, New York, USA. CCR, CC-chemokine receptor; HEC, high endothelial cell; LFA1, lymphocyte function-associated antigen 1.

The intrinsic binding function of HEVs is also enhanced approximately twofold in LPS- or turpentine-induced mouse models of fever, as well as during the exposure of mice to fever-range whole-body hyperthermia[136,137,140–142] (FIG. 3a). As in lymphocytes, maximal enhancement of HEV adhesion requires sustained temperature elevation (more than 6 hours)[136,137,140–142], recapitulating the extended time-frame of physiological fever responses. Chen *et al.*[140,141] visualized lymphocyte interactions in mouse HEVs using intravital microscopy (FIG. 3b), together with quantitative image analysis of trafficking molecules, to pinpoint the thermally responsive trafficking mechanisms in HEVs. Thermal stress does not alter the ability of HEVs to support rolling, nor does it change the intraluminal density of the prototypical rolling molecules PNAD or MADCAM1 (REFS 140,141). Instead, exposure to febrile temperatures profoundly increases the ability of HEVs to support the stable arrest of lymphocytes, and this can be attributed to heat-induced increases in the intravascular density of CCL21 and ICAM1 (REFS 140,141) (FIG. 3a). Notably, the level of HEV adhesiveness and ICAM1 expression induced by thermal stress is equivalent to that observed in response to the potent pro-inflammatory cytokine TNF[140]. Thermal upregulation of CCL21 and ICAM1 expression in HEVs is consistent with the known concentration-dependent roles of these molecules in augmenting LFA1 affinity (~10,000-fold), thereby supporting stable adhesion of lymphocytes within vessel walls[145,146]. In addition, increases in ICAM1 expression in response to hyperthermia probably promote LFA1-dependent transendothelial migration in HEVs and the formation of ICAM1-dense adhesive patches that guide lymphocyte diapedesis into underlying tissues[133,147,148].

Once lymphocytes gain entry into lymphoid organs there is evidence that their ability to respond to stimulatory signals is also enhanced by febrile temperatures. Direct exposure of T cells to fever-range hyperthermia increases their proliferation in response to mitogens[149,150]. Furthermore, in both *in vitro* and *in vivo* models of antigen-driven T cell activation by APCs, thermally treated $CD8^+$ T cells show greater differentiation towards an effector phenotype, with pronounced L-selectin downregulation, enhanced cytotoxic function and increased production of IFNγ[151,152] (FIG. 3a). Enhanced stimulation of naive $CD8^+$ T cells is aligned with temperature-dependent activation of protein kinase Cβ (PKCβ), prolonged stable contacts with APCs and transient clustering of components of the immunological synapse (the T cell receptor (TCR) β-chain and CD8) in cholesterol-enriched microdomains[151,152]. Similar heat-induced changes in membrane fluidity and macromolecular clustering in the plasma membrane occur in $CD4^+$ T cells that reduce the requirement for CD28 stimulation for IL-2 production[153]. These findings suggest that febrile temperatures lower the threshold for T cell signalling and effector T cell differentiation by pre-associating the signalling components of the TCR complex.

IL-6 is a thermally sensitive effector of trafficking. Investigation into the mechanisms underlying thermal regulation of trafficking led to the unexpected discovery that the same pyrogenic cytokine that is responsible for inducing fever — namely, IL-6 (REFS 135,137,138) — also controls both lymphocyte and endothelial adhesion[132,138–140,142]. The thermal response further depends on a second soluble factor, the soluble form of IL-6Rα (sIL-6Rα), which acts cooperatively with IL-6 and the membrane-anchored gp130 signal transducing molecule through a well-defined mechanism that is termed *trans*-signalling[138–140,154,155] (FIG. 4a). This thermally sensitive mechanism was identified *in vitro* and *in vivo* using recombinant soluble gp130 (REFS 138,140), which is a competitive antagonist of IL-6 *trans*-signalling but which does not affect classical signalling that involves membrane-anchored IL-6Rα[154,156].

In lymphocytes, the MEK1–ERK1/ERK2 signalling pathway, but not the p38 or JUN N-terminal kinase (JNK) pathways, operates downstream of IL-6–sIL-6Rα *trans*-signalling in response to heat[138]. This promotes L-selectin interactions with actin-based cytoskeletal scaffolding elements, thereby enhancing its apparent tensile strength (FIG. 4b). IL-6-induced activation of signal transducer and activator of transcription 3 (STAT3) also occurs in lymphocytes in response to thermal stress[138], although it is not known whether this contributes to lymphocyte adhesion or delivers survival signals[157,158] that aid the expansion of populations of effector lymphocytes within lymphoid organs. Consistent with the evolutionary conservation of the febrile response, L-selectin adhesion is induced by fever-range temperatures through a common IL-6 *trans*-signalling mechanism in animals representing four taxa of jawed vertebrates that includes endothermic mammals (for example, human, rodent, dog, cow, tiger, elephant and rhinoceros) and birds (chicken), as well as ectothermic amphibians and fish[134,135,137–139]. These observations strongly suggest that conservation of IL-6-regulated lymphocyte trafficking mechanisms over hundreds of millions of years of evolution confers a survival benefit during fever.

Ligation of gp130 by IL-6–sIL-6Rα also upregulates the intravascular density of ICAM1 in HEVs during heat treatment of mice[132,140] (FIG. 4b). The dual requirement for IL-6 and sIL-6Rα for ICAM1-dependent trafficking in HEVs during thermal stress is in line with the prevailing view that endothelial cells generally lack membrane-anchored IL-6Rα and thus are refractory to IL-6 unless sIL-6Rα is available[132]. STAT3 signalling and MEK1–ERK1/ERK2 signalling have been implicated in the transcriptional regulation of ICAM1 (REF. 132) and thus are potential mediators of the thermal response in HEVs. By contrast, CCL21 induction is not dependent on IL-6 *trans*-signalling[140], suggesting that an additional molecular pathway is induced by febrile temperatures.

One of the most intriguing findings to emerge from intravital imaging relates to the tight spatial regulation of IL-6–sIL-6Rα responses in vascular beds during thermal responses. In this regard, Chen *et al.*[140] showed that HEVs respond to IL-6 *trans*-signalling during thermal stress, but contiguous vascular segments that are not comprised of

high endothelial cells (HECs) are completely refractory to thermally induced IL-6 *trans*-signalling (FIG. 4b). Similarly, non-HEVs in other organs are not responsive to febrile temperatures, although heat shock (which occurs at temperatures greater than 43 °C) reportedly stimulates ICAM1 expression in normal vascular endothelium[137,140–142,159,160]. This restricted vascular response to physiological temperature elevation is proposed to maintain the focal trafficking of lymphocytes at HEVs in lymph nodes and Peyer's patches that are located throughout the body, thus maximizing their opportunity to scan pathogen-derived antigens from peripheral sites of infection[137,140,141].

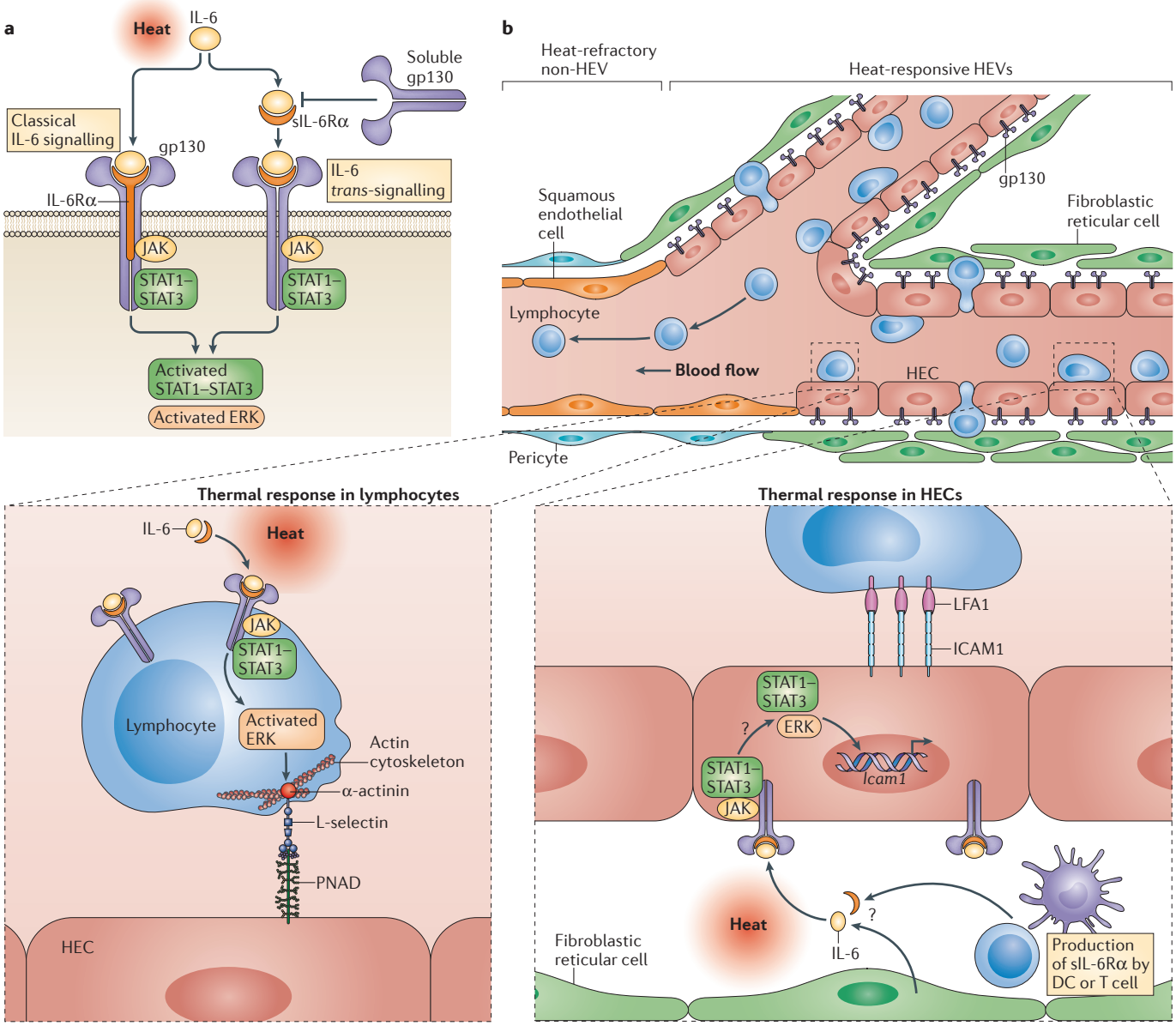
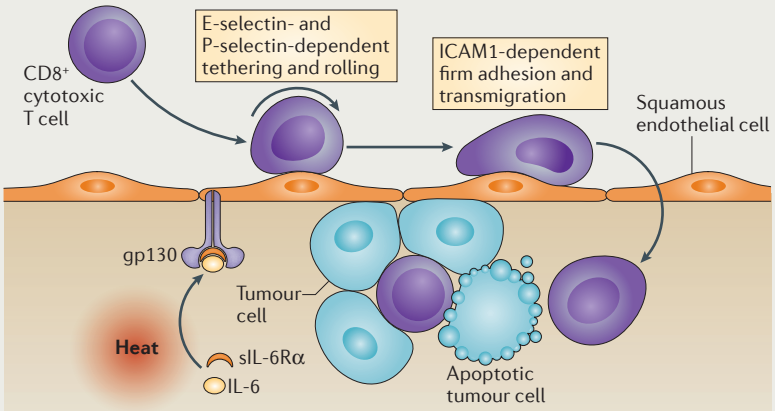


Figure 4 | **Thermal stress acts through IL-6 *trans*-signalling to improve lymphocyte trafficking into lymph nodes. a** | Heat-dependent interleukin-6 (IL-6) *trans*-signalling is initiated by binding of the soluble form of the IL-6 receptor-α subunit (sIL-6Rα) to both IL-6 and membrane-anchored gp130 (REFS 154,155). Soluble gp130 functions as a selective antagonist of IL-6 *trans*-signalling and downstream activation of canonical signalling pathways — mediated by Janus kinase–signal transducer and activator of transcription (JAK–STAT) and MEK1–extracellular signal-regulated kinase 1 (ERK1)/ERK2 — but does not interfere with classical signalling by membrane-anchored IL-6Rα and transmembrane gp130 (REF. 156). **b** | Febrile temperatures act on lymphocytes and high endothelial cells (HECs) to improve lymphocyte trafficking exclusively across high endothelial venules (HEVs) in lymph nodes. Vessel segments immediately proximal to HEVs are refractory to thermal treatment, which may reflect the lower expression of gp130 by squamous endothelial cells that line non-HEVs[162]. Fever-range temperatures act directly on lymphocytes through IL-6 *trans*-signalling to stimulate the MEK1–ERK1/ERK2 signalling pathway, promoting L-selectin adhesion and intermolecular interactions between the actin-based cytoskeleton, α-actinin and the cytoplasmic tail of L-selectin[138] (left inset). IL-6 *trans*-signalling upregulates the intravascular density of intercellular adhesion molecule 1 (ICAM1) in HEVs during heat treatment of mice, although the downstream signalling mediators remain unknown (right inset). Fibroblastic reticular cells that are in direct contact with HECs[165] are a possible source of the IL-6, and proximal dendritic cells (DCs) and T cells could provide the sIL-6Rα[138,164] required to enhance the adhesive properties of HEVs during thermal stress. LFA1, lymphocyte function-associated antigen 1; PNAD, peripheral node addressin.

The mechanism that maintains spatial resolution within venular segments over distances spanning the width of a single HEC (~30 μm)[161] remains to be resolved, but clues have emerged from recent transcriptional profiling of various cell subsets in lymphoid organs. HECs are distinguished from their normal endothelial cell counterparts by elevated expression of *Il6st* (which encodes gp130)[162], which could theoretically predispose them to be highly sensitive to IL-6–sIL-6Rα in the local milieu (FIG. 4b). Although the overall nodal concentrations of IL-6–sIL-6Rα are unchanged by thermal stress[140,163], heat could theoretically induce their synthesis by discrete cell populations or could lower the threshold for signalling in HECs. Fibroblastic reticular cells (FRCs) are a possible source of IL-6 during fever on the basis of their high expression of *Il6* mRNA relative to haematopoietic cells or vascular endothelial cells within skin-derived lymph nodes[164]. Unlike other vascular beds that are circumscribed by pericytes, HEVs are in direct contact with FRCs, and thus are optimally positioned to receive instructions from FRC-derived cytokines[74,75,165]. Of particular relevance is a report that IL-6 synthesis by fibroblasts can be induced by the heat-inducible transcription factor HSF1 (REF. 166). The sIL-6Rα necessary for *trans*-signalling is probably provided by neighbouring leukocytes, including DCs, monocytes and/or T cells[138,164]. Recent studies have shown that febrile temperatures can also act through IL-6 *trans*-signalling to augment the recruitment of cytotoxic $CD8^+$ T cells across tumour-associated vessels[142]. These studies are highly relevant to the use of thermal medicine as an adjuvant for cancer immunotherapy (BOX 2) and raise the possibility that fever could invoke similar mechanisms to amplify effector T cell trafficking at sites of infection.

Box 2 | **Thermal therapy and cancer**

Thermal therapy is administered at a wide range of temperatures for cancer treatment. High temperature focal hyperthermia (>45 °C) and ablation therapy (>70 °C) directly destroy cancer cells and can indirectly boost antitumour immunity, whereas moderate hyperthermic therapy (38–42 °C) is mainly used in an adjuvant setting to target the tumour microenvironment[206–208]. Temperature effects on blood flow, vascular permeability, interstitial pressure and hypoxia are implicated in enhanced chemosensitization and radiosensitization in patients with cancer treated with hyperthermia[209–215]. Thermal therapy also holds promise for improving the delivery of chemotherapeutic drug cargo by heat-sensitive liposomes[216]. Recent preclinical studies suggest that the immunostimulatory activities of febrile temperatures can be exploited therapeutically in combination with promising cancer treatments. Emerging immunotherapies such as dendritic cell (DC) vaccination, adoptive transfer of *ex vivo*-activated T cells or checkpoint blockade inhibitors (for example, drugs targeting cytotoxic T lymphocyte protein 4 (CTLA4) and programmed cell death protein 1 (PD1)) have shown benefit in generating antitumour immunity[217–220]. Notably, the efficacy of DC vaccines in patients with advanced melanomas or mouse tumour models is substantially improved with the use of hyperthermia as an adjuvant therapy[221,222]. Moreover, fever-range thermal therapy overcomes impediments to trafficking in mouse tumour vessels through an interleukin-6 (IL-6) *trans*-signalling mechanism that involves IL-6 binding to soluble IL-6 receptor subunit-α (IL-6Rα) and heat-induced gp130 on tumour endothelial cells. This, in turn, stimulates E-selectin- and P-selectin-dependent rolling and intracellular adhesion molecule 1 (ICAM1)-dependent firm adhesion of adoptively transferred cytotoxic $CD8^+$ T cells (see the figure). Increased T cell entry into tumours is further linked to improved antitumour immunity and delayed tumour growth[142]. The antitumour immune effects of IL-6 that are induced by thermal therapy are counterintuitive in light of substantial evidence that IL-6 signalling exerts pro-tumorigenic activities by stimulating the survival and proliferation of tumour cells, as well as angiogenesis[155,223]. Together, these studies highlight a unique role for thermal therapy in modulating the tumour microenvironment that can be co-opted to increase the efficacy of diverse anticancer therapies.



A return to homeostasis

The immune response must be tightly regulated to avoid excessive tissue damage after infection. By extension, it makes sense that the effects of febrile temperatures on the immune system are also temporally regulated during the resolution phase of inflammation, although a full picture of the underlying mechanisms is yet to emerge. One example is the rapid restoration of lymphocyte trafficking in HEVs to basal levels within 6 hours following cessation of fever-range hyperthermia[134,137,141]. Normalization of HEVs is mediated by zinc-dependent metalloproteinases that cleave endothelial ICAM1 while sparing other trafficking molecules (such as PNAD)[141], although it is not known whether heat stimulates the catalytic activity of these enzymes. In line with a potential anti-inflammatory role of hyperthermic temperatures, heat shock (42 °C for 15 minutes) has been found to blunt leukocyte adhesion within vessels if administered 2 days before the intravascular delivery of the neutrophil attractant FMLP *in vivo*[167].

Although febrile temperatures initially increase the production of pro-inflammatory cytokines by macrophages at sites of inflammation[59–61,95,96], there is also evidence that thermal stress dampens cytokine synthesis once macrophages become activated. This sequence of events is analogous to natural fever, which often occurs after macrophages and other innate immune cells initially encounter PAMPs. In this regard, human monocyte-derived macrophages with an activated phenotype produce less IL-1β, IL-6 and TNF when exposed to febrile temperatures than heat-inexperienced cells[95,96,168–170]. Heat reduces the transcription of pro-inflammatory cytokines through repressive activities of HSF1, together with diminished recruitment of NF-κB to the promoter regions of cytokine-encoding genes, and also lowers cytokine mRNA stability[171–173]. Thermal treatment of LPS-activated macrophages also seems to dial down

inflammation by inhibiting the release of the inflammatory DAMP high mobility group protein B1 (HMGB1), which is a ligand for TLR2 and TLR4 (REFS 170,174). Inhibition of HMGB1 release prevents the subsequent activation of NF-κB, which controls the synthesis of pro-inflammatory cytokines in innate immune cells[169,170,174]. The idea that heat can dampen an ongoing pro-inflammatory condition *in vivo* has recently been tested in a mouse model of collagen-induced arthritis[175]. Mice exposed to fever-range hyperthermia had less joint damage, which was correlated with a reduction in serum TNF levels and increased IL-10 production in inflamed joints. Collectively, these findings suggest that strategic temperature shifts contribute to a biochemical negative feedback loop that protects tissues against damage from excessive cytokine release following infection.

Thermogenesis and adrenergic signalling

Neural components of the thermoregulatory system continuously monitor temperature changes throughout the body and initiate integrated responses that either increase internal heat content (for example, through thermogenesis in brown adipose tissue) or increase the dissipation of heat (for example, following intense exercise)[8]. Given the homeostatic importance of thermoregulation it is all the more remarkable that fever has been so long maintained in evolution, as natural thermoregulatory signals must be suppressed in order to increase body temperature. Although the examples discussed above demonstrate that the immune system is responsive to elevated temperatures, new studies have revealed that this system is also highly sensitive to the metabolic stress that is associated with thermogenesis. Emerging evidence strongly supports a direct role for cold stress-induced noradrenaline production and the interaction of noradrenaline with β-adrenergic receptors on immune cells as a major mechanism for immune modulation by environmental cold stress. It is well established that noradrenaline-driven stimulation of β-adrenergic receptors is crucial for the release of additional heat from mitochondria in brown adipose tissue during cold stress to maintain a normal core body temperature[176,177]. Moreover, the ubiquitous presence of β-adrenergic receptors has been observed on the surface of immune cells, and there is a growing appreciation of the functional consequences of signalling through these receptors[178–180]. Even more recent studies have demonstrated a crucial role for β-adrenergic receptor signalling by noradrenaline for the control of lymphocyte egress from lymph nodes and the modulation of cytokine production and proliferation in $CD8^+$ memory T cells[181,182].

These parallel lines of research have now been joined in studies that demonstrate marked alterations in immune cell activity during cold stress. Nguyen *et al.*[183] discovered that cold stress stimulates IL-4- and IL-13-driven differentiation of macrophages in brown adipose tissue towards an 'alternative activation' programme that leads to their production of noradrenaline (FIG. 5a). Surprisingly, data obtained using various knockout mice (deficient in IL-4, IL-13, STAT6 or IL-4 receptor) revealed that the noradrenaline produced by these macrophages is crucial for maintaining sufficient thermogenesis in the face of cold stress[183,184]. Kokolus *et al.*[184] further demonstrated that DCs from cold-stressed mice that have a normal body temperature owing to increased thermogenesis exhibit a reduced ability to stimulate T cells. Cold stress is also associated with accelerated tumour growth in murine models, which reflects enhanced tumour cell survival pathways and a shifted balance towards an immunosuppressive environment; this environment is associated with increased numbers of myeloid-derived suppressor cells and intratumoural regulatory T cells together with reduced numbers of $CD8^+$ effector T cells[184–186] (FIG. 5b).

An intriguing aspect is that the presence of cancer creates a notable heat-seeking behavioural response in animals[184]. These data support the conclusions drawn by Romanovsky and colleagues who have contended that endothermic animals, including humans, exhibit heat-seeking behaviour even before other fever-generating symptoms occur[9,10]. Findings in this exciting area contribute additional molecular detail to the fundamental role of temperature stress in influencing the functional balance between both arms of the immune system[187,188].

Concluding remarks and future directions

The evolutionary conservation of the fever response over millions of years is in line with its protective role: the survival benefit conferred on the host outweighs the metabolic cost of elevating core body temperatures during infection. Cellular components of the immune system have emerged as central components that actively drive fever induction in addition to serving as thermally sensitive effectors. Moreover, the complexity of the molecular pathways that coordinate a febrile response is mirrored by the diverse cell types that are affected by hyperthermic temperatures: these include DCs, macrophages, NK cells, neutrophils, B cells, T cells and vascular endothelial cells. The picture that emerges is one in which febrile temperatures serve as a systemic alert system that broadly promotes immune surveillance during challenge by invading pathogens. Furthermore, mechanistic insight into the immune-protective nature of fever has opened up new avenues to exploit the immunostimulatory activities of thermal stress in the context of cancer therapy.

Fundamental questions remain regarding the nature of the temperature-sensing machinery that triggers changes in immune cell behaviour. HSF1-regulated HSPs are strong candidates in view of their rapid induction even at the relatively modest temperature elevation (ΔT ~1–4 °C) that accompanies fever[26,122,189–191]. Also intriguing are reports that HSF1 regulates additional genes that are relevant to the induction and/or effector phases of fever, including *IL6* and *COX2* (REFS 166,192). Notably, HSP90 and the JAK1–JAK2–STAT3 signalling axis triggered by IL-6 are participants in a feedforward loop: IL-6–STAT3 signalling stimulates HSP90 production, and JAK2 and STAT3 are established client proteins that are chaperoned by HSP90 (REFS 193–197). Thus, it is tempting to speculate that the induction of HSP90 or other HSPs by febrile temperatures lowers the threshold for IL-6 signalling. Additionally, a class of temperature-sensing transient receptor potential (TRP) cation channel

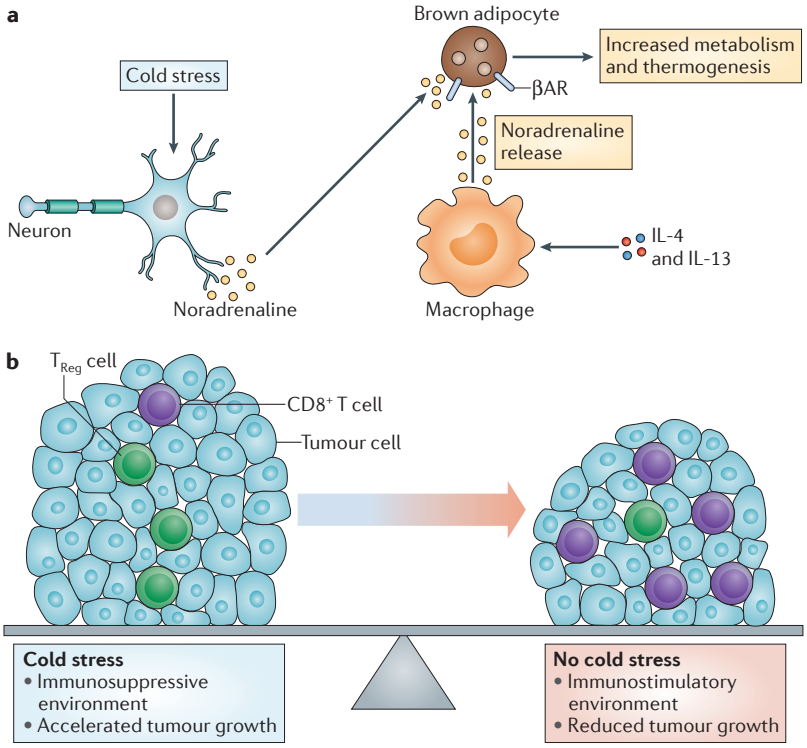


Figure 5 | **Cold stress stimulates nerve-driven modulation of thermogenesis and antitumour immunity. a** | Exposure to cold stress drives the release of neurotransmitters, such as noradrenaline, by neurons. This initiates the interleukin-4 (IL-4)- and IL-13-driven 'alternative activation' programme of differentiation in macrophages, resulting in the additional production of noradrenaline, which stimulates β-adrenergic receptors (βARs) that are expressed on brown adipocytes, thus driving thermogenesis[183]. **b** | Cold stress in tumour-bearing mice maintained at standard housing temperatures (20–26 °C) tilts the balance towards an immunosuppressive local tumour microenvironment. This is characterized by a substantial increase in the number of intratumoural regulatory T (T_{Reg}) cells, and a concomitant decrease in the number of $CD8^+$ T cells when compared with tumours that develop in mice housed under thermoneutral ambient temperature (30–31 °C)[184]. Tumour cell survival and tumour growth are also accelerated by cold stress[184–186].

proteins expressed on immune cells and endothelial cells are likely to coordinate responses to febrile temperatures and inflammatory cytokines, such as IL-6 and lipid mediators[26,198,199]. There are also unanswered questions regarding the mechanisms that underlie the spatial regulation by IL-6 during fever induction and lymphocyte trafficking in HEVs. Although brain endothelial cells and HECs are predicted to be the main targets of IL-6, the contributions of intermediary cells have not been excluded.

Another unanswered question is whether febrile temperatures mobilize innate and adaptive immune cells to sites of infection. Observations that the administration of fever-range hyperthermia is effective in boosting E-selectin-, P-selectin- and ICAM1-dependent trafficking of cytotoxic $CD8^+$ T cells in tumour tissues[142] raise the strong possibility that similar mechanisms are triggered in infected tissues during fever. Similarly to CXCL8, several inflammatory chemokines that recruit NK cells, $CD4^+$ and $CD8^+$ T cells, and monocytes (such as CXCL9, CXCL10, CXCL11 and CXCL12), contain putative HSF1-binding sites within their gene promoters and, consequently, may be induced by thermal stress[26]. An important caveat is that information regarding the cytokine circuitry (for example, IL-1, IL-6 and RANKL) leading to fever, as well as the impact of temperature on immune function, is mostly based on experimental models using LPS or fever-range hyperthermia as surrogates for pathogen-induced fever. Although these studies provide insight into the mechanistic underpinnings of immune regulation by temperatures within the febrile range, lessons learned from studies of thermogenesis[183–185] indicate that overall temperature sensing (cold or hot) in the absence of disease can have unexpected outcomes in innate and adaptive immunity. The next frontier will be to establish whether the same mechanisms that have been identified during the challenge of healthy animals with LPS or fever-range hyperthermia also operate during febrile responses to pathogens.

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Competing interests statement
The authors declare no competing interests.