CYTOKINES AND FEVER
Bruno Conti, Iustin Tabarean, Cristina Andrei and Tamas Bartfai

Harold L. Dorris Neurological Research Center, Department of Neuropharmacology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA

TABLE OF CONTENTS
1. Abstract
2. Introduction
3. Cytokines as endogenous pyrogenic and antipyretic agents
   3.1. Interleukin 1 beta and the Interleukin 1 system
   3.2. Tumor Necrosis Factor
   3.3. Interleukin 6
   3.4. Interleukin 1Receptor Antagonist
   3.5. Interleukin 10
4. LPS and TLR: confluence of IL-1 and LPS signaling
5. Actions of cytokines on the hypothalamic thermoregulation
   5.1. Actions of peripheral cytokines
   5.2. Actions of cytokines formed in the CNS
6. Effects of pyrogenic cytokines on the firing rate of neurons in the preoptic area/anterior hypothalamus area (POA/AH)
7. Conclusion
8. Acknowledgements
9. References

1. ABSTRACT

Cytokines are highly inducible, secreted proteins mediating intercellular communication in the nervous and immune system. Fever is the multiphasic response of elevation and decline of the body core temperature regulated by central thermoregulatory mechanisms localized in the preoptic area of the hypothalamus. The discovery that several proinflammatory cytokines act as endogenous pyrogens and that other cytokines can act as antipyretic agents provided a link between the immune and the central nervous systems and stimulated the study of the central actions of cytokines. The proinflammatory cytokines interleukin 1 (IL-1), interleukin 6 (IL-6) and the tumor necrosis factor alpha (TNF) as well as the antiinflammatory cytokines interleukin 1 receptor antagonist (IL-1ra) and interleukin 10 (IL-10) have been most investigated for their pyrogenic or antipyretic action. The experimental evidence demonstrating the role of these secreted proteins in modulating the fever response is as follows: 1) association between cytokine levels in serum and CSF and fever; 2) finding of the presence of cytokine receptors on various cell types in the brain and demonstration of the effects of pharmacological application of cytokines and of their neutralizing antibodies on the fever response; 3) fever studies on cytokine- and cytokine receptor- transgenic models. Studies on the peripheral and the central action of cytokines demonstrated that peripheral cytokines can communicate with the brain in several ways including stimulation of afferent neuronal pathways and induction of the synthesis of a non cytokine pyrogen, i.e. PGE2, in endothelial cells in the periphery and in the brain. Cytokines synthesized in the periphery may act by crossing the blood brain barrier and acting directly via neuronal cytokine receptors. The mechanisms that ultimately mediate the central action of cytokines and of LPS on the temperature-sensitive neurons in the preoptic hypothalamic region involved in thermoregulation, directly or via second mediators, remain to be fully elucidated.

2. INTRODUCTION

Fever is an induced increase of the body core temperature caused by a regulated elevation of the temperature set point evoked by changes in the neuronal activity of components localized in the preoptic area of the hypothalamus. Because fever can be induced by a multitude of substances administered peripherally ranging from inorganic to organic compounds or microbial and mammalian proteins, the existence of a common endogenous fever mediator was postulated. Eventually, the much sought “endogenous pyrogen” was found to be identical to the leukocyte activating factor (LAF), now most commonly known as interleukin 1 (IL-1), one of the prototypic proinflammatory cytokines (see (1) for review). This finding led the way to the identification of the pyretic and antipyretic properties of several cytokines and to the investigation of their role as an endogenous mediator of the febrile response.

In the present review, we summarize the experimental evidence demonstrating the action and the effects on fever of some of the most extensively investigated cytokines. While several cytokines have been investigated for their pyrogenic action, including ciliary neurotrophic factor, interleukin 2, interleukin 12 and interferon alpha, we will focus on the action of the three major proinflammatory cytokines: interleukin 1beta (IL-1beta), interleukin 6 (IL-6), Tumor Necrosis Factor alpha (TNF); and on those of the antiinflammatory cytokines: IL-
Cytokines are intercellular signaling proteins mainly produced by immuno-competent cells. Their primary function is the regulation and the coordination of immune responses. Expression of several cytokines and their receptors were also demonstrated in the central nervous system where they are produced by glial or neuronal cell types. In the CNS, cytokines are believed to function as possible trophic factors, which can directly affect neuronal function and which also participate in local inflammatory processes. Several cytokines, including IL-1beta, IL-6, TNF, IL-1ra and IL-10 were also demonstrated to have pyretic or antipyretic effects. Such conclusions came primarily from three different experimental evidences demonstrating: a) association between the levels of these cytokines in the serum and/or in the cerebrospinal fluid (CSF) and fever; b) the effects of peripheral and central pharmacological application of the cytokine on core body temperature; c) cytokine action in studies on transgenic animals overexpressing or null mutated for a specific cytokine or its receptor; d) cytokine action in experiment employing specific neutralizing antibodies; e) autonomic and behavioral responses in establishing and maintaining fever response to cytokines and other pyrogens were also instrumental. Although not a major mechanism of thermoregulation in homeothermic animals they can regulate core body temperature by combined use of both autonomic and behavioral mechanism: i.e. the animals may seek out cooler or warmer places during the different phases of fever response to conserve the body’s energy reserve.

In evaluating the effects of cytokines, it is important to consider not only the experimental approach utilized, but also some peculiar features of these protein molecules. Cytokines are among the most highly inducible proteins known. Their expression level can go from $10^{-15}$–$10^{-12}$ M, barely detectable levels, to $10^{-9}$–$10^{-7}$ M in a matter of 30-90 minutes in response to bacterial, viral, inflammatory or stress stimuli. In addition, cytokines can regulate each others production. For instance, IL-1beta is one of the most potent inducers of TNF as well as of IL-1ra and TNF is a powerful stimulator of IL-1beta production; finally both IL-1beta and TNF potently induce IL-6 (3, 4). Thus, while proinflammatory cytokines are normally synthesized and active in response to stimulation (i.e. bacterial infection), their rapid and potent cross-induction renders the understanding of their hierarchical action difficult to investigate. In addition, their relevance with respect to the fever response also depends on the possible presence and dose of bacterial or viral pyrogens. Lipopolysaccharide (LPS) from Gram negative bacteria can in fact activate the same intracellular signaling pathway as IL-1 and thus bypass its action if present at high dose (5).

Figure 1 illustrates the confluence of the IL-1 and LPS signaling via their respective Toll family receptor.
Cytokines and fever

3.1. Interleukin 1beta and the Interleukin 1 System

The members of the IL-1 family, IL-1beta, IL-1alpha and the receptor antagonist IL-1ra, are probably the most extensively investigated cytokines. In humans, the genes encoding for these three proteins are located in a cluster on chromosome 2 (2q14) (6-8) mainly membrane associated, while. IL-1alpha is secreted after maturation of its precursor form (pro-IL-1beta) by IL-1beta caspase 1 (or ICE, interleukin 1 Converting Enzyme) (9, 10). IL-1ra is a secreted protein acting as a negative regulator of IL-1alpha and IL-1beta actions. IL-1alpha, IL-1beta and IL-1ra all bind the same IL-1 receptors: the type I and Type II IL-1 receptors. Type I IL-1 receptor (IL-1RI) is a member of the Toll receptor family (11-15). The heterodimeric IL-1RII complex is composed of the IL-1 binding protein receptor (IL-1R) and the IL-1R accessory protein (IL-1RAcP) responsible for signal transduction (16, 17). Binding of IL-1 to the IL-1RI leads to structural changes allowing docking of the IL-1R-AcP, a necessary event for the recruitment/activation of the Myeloid Differentiation Primary Response Protein (MyD88), IL-1 Receptor-Associated Kinase (IRAK), TNF receptor-associated factor (TRAF) signaling pathway ultimately leading to activation of the Nuclear Factor Kappa B (NF-kB) (18-20). A second type of IL-1 receptor (IL-1RII), acts as a “decoy” molecule binding IL-1beta with high affinity yet it fails to activate the signaling cascade (21, 22). LPS up-regulates the expression of IL-1alpha, IL-1beta and of the IL-1RII, while causing a rapid decrease, followed by inhibition of mRNA expression, of the IL-1RII (23). IL-1beta level is elevated in plasma and cerebrospinal fluid (CSF) of several species following peripheral application of LPS (24), turpentine (25) and zymogen (26) leading to fever. Injection of IL-1beta induces fever in rodents (27), rabbit (28) and humans (29). A direct role of the IL-1 system in mediating fever response is also confirmed by the effects of IL-1ra pretreatment or overexpression, both reducing the IL-1beta induced fever (30-32). Such effects may be systemic or local since fever can develop even in the absence of detectable circulating levels of IL-1 beta. Similarly, IL-1RI as well as IL-1RAcP null mutations prevent IL-1beta- induced fever (33, 34). IL-1-induced fever can be blocked by antibodies to IL-1 beta injected into the hypothalamus (35) or by pretreatment with cyclooxygenase-1 (COX1)-2 inhibitors (36) and it is dependent on the expression of the EP3 type prostanoid receptor for PGE2 indicating that IL-1beta acts upstream of PGE2 (37). IL-1 can regulate the fever response also through transcriptional regulation of the production of other pyrogenic (TNF and IL-6) (32) and antipyretic cytokines (IL-1ra and IL-10) from macrophages, endothelial cells and microglia (38-40). IL-1beta also mediates a number of fast biochemical events in a transcription-independent manner. Protein phosphorylation (mainly on Ser/Thr, but sometimes on Tyr residue) as well as protein phosphatase activation can be observed within 5 min of IL-1beta treatment in several cell types such as macrophages. These events are at least in part mediated by lipid mediators. For instance, one possible early mediator of IL-1beta transcription-independent action was proposed to be ceramide, released following activation of N-SMase (41-43). In cortical synaptosomes, ceramide elevates [Ca2+]i, in a p42 MAP kinase-dependent manner (44). In rat pinealocytes, ceramide mediates the inhibitory effect of IL-1beta on the L-type Ca2+ channel current, through the activation of a tyrosine kinase (45). In the same system it was also shown that ceramide reduces the outward K+ current predominantly by inhibiting I(KCa), in a PKC dependent manner (46).

More recently a feedback mechanism of febrile temperature elevation on the LPS-induced release of IL-1beta was proposed by Boneberg and Hartung (47) showing that IL-1beta secretion is impaired at temperatures higher than 38°C.

3.2. Tumor Necrosis Factor

The tumor necrosis factor alpha (TNF-alpha or TNF) is the principal mediator of acute inflammation in response to Gram-negative bacteria. It is mainly produced by LPS-activated mononuclear phagocytes (48), but can be secreted also by antigen-stimulated T cells, natural killer and mast cells (49, 50). Human TNF gene is located on the short arm of chromosome 6 (51). TNF is synthesized as a non-glycosylated membrane protein with an intracellular N-terminus and a large extracellular C-terminus and it is expressed on the membrane as a homotrimer. Each subunit is cleaved in a 17Kd fragment by the membrane-bound metalloprotease tumor necrosis factor alpha converting enzyme (TACE) producing the secreted form circulating as a homotrimer of 51Kd (52), a pyramid-shaped complex that can simultaneously bind more than one TNF receptor (53). TNF interacts with two transmembrane signaling receptors, type I (p55) and type II (p75) (54, 55); a third soluble receptor exists that act as an antagonist of TNF activity (56).

The role of TNF in fever response is not clear as this molecule has been shown to have both pyrogenic and antipyretic effects. Injection of TNF in human and animals is followed by fever response suggesting its pyrogenic action (57-59). As TNF induces IL-1 it is possible that this is one of the mechanisms that lead to a TNF induced elevation in body core temperature (1, 60). Intracerebroventricular injection of a high dose of LPS leads to an elevation of TNF and IL-6 levels in the cerebrospinal fluid (61). Consistently, pretreatment with TNF antiserum has been shown to inhibit turpentine or LPS induced fever (62-64), but not adenovirus induced fever (65). However, rats treated with low non-pyrogenic dose of TNF, showed a reduced fever upon LPS injection; while the fever response was enhanced by injection of soluble TNF receptor suggesting antipyretic action of TNF (66). Similarly, TNF enhanced and sTNFR attenuated the early hypothermic response induced by LPS (67). Experiments with TNFR null mice showed that LPS induced comparable fever response in the TNF-KO and the wild type mice. However, when challenged with high dose of LPS, the TNFR KO mice showed an exacerbated fever response rather than a depressed febrile response to LPS; such response was accompanied by a reduced plasma level of IL-10 (68). Moreover, circulating levels of TNF, in response to LPS, are enhanced by COX-2 inhibitors (69).

It has been shown that LPS produces a dual body temperature response, in which initial hypothermia precedes fever. This “dual temperature response” clearly depends on the injected LPS dose and on the ambient
temperature (see for example: 70, 71). Serum TNF levels rise during the initial phase of the LPS-induced hypothermia. Inhibitors of COX-2 completely abolish the LPS-hypothermia, resulting in an acceleration of the fever phase, leaving unaltered the peak and plateau phases of fever (72). It is thus possible that TNF is responsible for the initial hypothermia observed in mice following bacterial infection, as often TNF induces a dual phase fever response leading to mortality. Augmenting the dose of IL-1beta injected, hypothermia becomes more profound (73). Exogenous IL-1beta, IL-1alpha or LPS induce hyper-responsive fevers in the IL-1beta-deficient mice (74). This dual role of pyrogens may be explained by feedback mechanisms that are activated by the pyrogenic cytokine itself and by prostaglandin on cytokine formation (69) to control the fever response. The exacerbated fever in the TNFR knockout mice could also be due to similar feedback mechanisms being disturbed.

3.3. Interleukin 6

Interleukin 6 (IL-6) is an important mediator of the host response to disease. The human gene of IL-6 has been localized on chromosome 7p15-p21 (75) and is transcribed in different cell types, including monocytes, synovioocytes, vascular endothelial cells and fibroblasts upon stimulation with different infective and inflammatory stimuli. In the CNS IL-6 is synthesized by glial cells and neurons (76, 77). Emerging evidence suggests that IL-6 possesses neurotrophic properties, indicating its possible critical role as a physiological neuromodulator regulating diverse brain functions (78). IL-6 binds to a specific surface receptor complex consisting of specific cytokine binding subunits, IL-6R alpha chain and a signal transducing protein gp130 (79, 80).

Together with IL-1beta, IL-6 is considered to be the major endogenous pyrogen (81). Fever is accompanied by the appearance of IL-6 in the serum and the CSF (61, 82). Injection of LPS into a subcutaneous air pouch in rats evokes an increase in the concentration of bioactive IL-6 in the plasma (83) and a rise in body temperature that is abolished by pretreatment with IL-6 antisera (84). However, injection of even high dose of IL-6 alone did not induce an elevation in body core temperature unless co-administered with IL-1beta, either at pyrogenic or non-pyrogenic dose (32, 84, 85). In IL-6 null mice, neither intraperitoneal administration of low dose of LPS (50 microg/kg) or IL-1beta, nor intracerebroventricular injection of IL-1beta induced a fever response (86). An elevation of body core temperature was instead evoked (rescued) by intracerebroventricular injection of IL-6 (86, 87). These results suggest that central IL-6 is a necessary component of the fever response to both endogenous (IL-1beta) and exogenous (LPS) pyrogens in mice and that IL-6 acts downstream from both peripheral and central IL-1beta. Similar to IL-1, the fever response induced by central injection of IL-6 can be suppressed by inhibiting COX-2 activity (87, 88).

3.4. Interleukin 1Receptor Antagonist

Interleukin 1 receptor antagonist (IL-1ra) is the endogenous antagonist of IL-1 agonist: IL-1alpha, IL-1beta. This cytokine binds to the IL-1RI receptor but fails to trigger IL-1 induced signaling even at very high concentrations, acting therefore as a bona fide receptor antagonist (89). As IL-1ra production is induced in a dose-dependent manner by IL-1alpha, IL-1beta and TNF, it seems to play an important role as an endogenous negative regulator of the action of these cytokines (38). Indeed, it was demonstrated that endogenous IL-1ra is produced locally at the site of inflammation to limit the fever response (90, 91). Injection of LPS in a subcutaneous pouch in rats induces a local rise of IL-1alpha and IL-1beta concentrations followed 2 hours later by an increase in IL-1ra level. Although neutralizing IL-1ra did not affect the maximum body temperature reached after LPS injection, it significantly prolonged the duration of the fever and was accompanied by a 3- to 4-fold increase of IL-1beta level (90). The role of IL-1ra was also investigated in the regulation of the firing rate of warm and cold sensitive neurons in the hypothalamus (discussed in further detail in the chapter “actions of cytokines on the hypothalamic thermoregulation”). In guinea pigs hypothalamic preoptic area slices, human recombinant IL-1beta reduced the firing rate of warm sensitive neurons, while increasing that of cold sensitive neurons and proving ineffective on thermally insensitive neurons. IL-1ra had no effect by itself on the firing rate of these neurons; rather it blocked the hIL-1 beta-induced changes in firing rate when given before the pyrogenic cytokine (92).

3.5. Interleukin 10

Interleukin 10 (IL-10), originally known as cytokine synthesis inhibitory factor (CSIF), is an antinflammatory cytokine and endogenous antipyretic produced by Th2 lymphocytes, monocytes, macrophages and other cell types (93). Human IL-10 is encoded by a single gene on chromosome 1 (1q32.1). IL-10 is active as a 37KDa homodimer secreted by a catecholamine via NF-variety of stimuli including endotoxins, TNF and NFκB or the Cyclic AMP-Response Element Binding protein/Activating Transcription Factor (CREB1/ATF) (94, 95). IL-10 receptor (IL-10R) is composed of two different chains, alpha and beta. Upon binding to IL-10, IL-10R activates the tyrosine kinases Jak1 and Tyk2, eventually leading to nuclear translocations of the transcription factors STAT1, 3 and 5 (96). The antinflammatory activity of IL-10 is mostly mediated by its effect on the inhibition of proinflammatory cytokines synthesis including IL-1, IL-6 and TNF. These effects are obtained by blocking NF-κB translocation, via inhibition of IKK (97), as well as NF-κB binding to the DNA (98).

Mice injected intraperitoneally with recombinant murine IL-10 or rats treated with IL-10 intracerebroventricularly are resistant to LPS-induced fever (99, 100). Consistently, IL-10 knockout mice responded to a low dose LPS with an exacerbated and prolonged fever, accompanied by elevated plasma level of IL-6; a high dose of LPS induces in IL-10 knockout mice a profound long lasting hypothermia, leading to enhanced mortality (99). An IL-10 mediated suppression of both Gram negative and Gram positive bacteria induced increase in IL-6 concentrations was reported by
Cytokines and fever

Cartmell et al., suggesting a role of IL-10 in defervescence (101).

4. LPS AND TLR: CONFLUENCE OF IL-1 AND LPS SIGNALING

Lipopolysaccharide (LPS), a major component of the Gram negative bacterial cell wall, is a potent pyrogen and strong inducers of proinflammatory cytokines. The discovery that LPS acts predominantly through the Toll-like Receptor 4 (TLR4) (102) sheds new light on the cellular mechanisms of fever. While LPS is a strong inducer of proinflammatory cytokines IL-1, IL-6, TNF etc., LPS induced fever still occurred in IL-1R knockout animals. It was at first thought that LPS could bypass IL-1 action by stimulating TNF (103), until it was demonstrated that fever following LPS administration still occurred in TNFp55/p75 receptor double knockout mice (104). LPS acts in the form of a complex with its binding protein (LBP) that binds to CD14, a membrane protein associated with a complex including TLR4. TLR4 is coupled to the MyD88 and signals through the IRAK/TRAF (i.e. Toll pathway); this is the same pathway that is activated by IL-1 upon its binding to the IL-1RI/IL-1RACp complex leading to NF-kB-dependent transcriptional changes including COX2 and iNOS induction (88, 105-108) (Figure 1).

It is evident that LPS can act directly on the fever response by activating the same signaling pathways as IL-1 does but through an independent receptor (the TLR4 receptor). These findings demonstrated that two major peripheral pyrogens, one endogenous (IL-1) and one exogenous (LPS), can induce fever response independently but through the same intracellular mechanisms (Toll signaling) thus converging most likely on the same cellular mechanisms involved in the central responses leading to fever.

5. EFFECTS OF CYTOKINES ON THE HYPOTHALAMIC THERMOREGULATION

The elevation of body core temperature that occurs during fever is centrally regulated by neurons in the preoptic region of the hypothalamus. IL-1, IL-6, TNF, IL-1ra and IL-10 can be produced in the periphery as well as in the CNS, it is thus important to distinguish their peripheral versus central actions.

5.1. Actions of peripheral cytokines

How can peripheral cytokines affect central mechanisms of fever? Several theories have been advanced, for review see (109, 110).

A. The existence of a carrier-mediated transport into the brain has TNF, IL-6 been reported for IL-1alpha, IL-1beta, (111-115) and also for IL-1ra (116), but not for IL-10 (117). This mechanism appears to be mediated by low capacity saturable transporters that could account for a modest elevation of central level of cytokine over an extended period of time, possibly sufficient to have a biological effect on the receptor in hypothalamic neurons. The "transporters" function in a way different from conventional receptors, in that cytokines are chaperoned from blood to the CNS rather than being degraded in the specialized endothelial cells composing the blood brain barrier (BBB). For TNF it has been demonstrated, using double knockout mice of p55 and p75 receptors, that the entry of the cytokine into the brain and the spinal cord is dependent on both receptors (118, 119). The saturable transport systems for IL-1, IL-6 and TNF are distinct molecular entities that are distinguishable from each other. However, these cytokine transport mechanisms seem to be too slow and easily saturable even at cytokine concentration much lower than those peripherally present (e.g. after LPS injection) to explain the rapid effects in the brain.

B. Cytokines might reach the brain through areas devoid of a BBB, as in the sensory circumventricular organs: particularly at the organum vasculosum laminae terminalis (OVLT) in the midline of the POA. There is evidence to indicate that endothelial cells in the OVLT bind circulating cytokines to cytokine receptors on their luminal surface. It has been shown that injection of LPS rapidly stimulated transcription of the IL-6R gene in the choroid plexus and the sensorial circumventricular organs (CVOs), including the OVLT, subformical organ, median eminence and area postrema (120); also the expression of TNF is induced in CVOs following peripheral LPS stimulation (121). Thus, peripheral cytokine activation of endothelial cells in circumventricular organs may result in the release of putative neuroregulators (cytokines released in the brain or PGE2) which then process the original signals inwardly to the POA (122-124), or the neurons in the OVLT might have synaptic projections to the neurons mediating thermoregulation. This hypothesis is supported by experimental evidence that cytokine or PGE2 application at the OVLT both evoke fever (123, 125), although the neuronal projections have not yet been mapped and some findings are not supportive of the OVLT mediated fever (126).

C. The action of peripheral cytokines on the central thermoregulatory circuit may be mediated by neuronal afferents (127-130). Several laboratories have shown that severance of the vagus nerve abrogates or lowers the amplitude of the fever response caused by intraperitoneal injection of IL-1beta (131-133) or LPS (134, 135) even if a dose related effectiveness has been proposed (136, 137). To date it is not known which of the vagal fibers carry the information relevant for the fever response. Yet, it was demonstrated that cytokine receptors present on peripheral nerve cells can influence neuronal activity indicating that cytokine effects on neuronal transmission via vagus nerve constitute a possible mechanism by which changes in cytokine levels in the periphery affect/evolve a fever response. Sensory neurons of the vagus nerve express receptors to IL-1 and prostaglandin E2 and circulating IL-1 stimulates vagal sensory activity via both prostaglandin-dependent and -independent mechanisms (138-140). Blatteis et al., have postulated that the pyrogenic message of peripheral LPS is conveyed very rapidly via vagal afferents to the nucleus tractus solitarius, where it passes to the A1/A2 noradrenergic cell groups, which transmit it to
Cytokines and fever

the anteroverentral third ventricle (AV3V)/POA region via the ventral noradrenergic bundle. The norepinephrine released in this site (141) stimulates the local release of PGE₂ (142), thus presumably triggering the febrile response. Vagotomy is a large and severe lesion in rodents. Yet, it was also demonstrated in rats that integrity of the vagus nerve is only associated with sickness behavior but not with hyperthermia/fever (143, 144).

D. Peripheral cytokines mediate induction of brain cytokines synthesis at the endothelial cells (145, 146). Endothelial cells possess receptors for proinflammatory cytokines such as IL-1 (147). Low dose of LPS and TNF mRNAdministered by peripheral injections induces IL-1beta expression only in the choroid plexus, the circumventricular organs and meninges, while septic doses of LPS induce global expression of proinflammatory cytokines in the brain (148, 149). Proinflammatory cytokines produced by these cells may serve as a signal for adjacent or more distant targets including neurons, endothelial and microglial cells. This mechanism is supported by evidence that intracerebroventricular administration of IL-1ra is able to diminish peripheral IL-1-induced fever (32). Intracerebroventricular injection of IL-1ra also significantly attenuates fever induced by LPS injection into a subcutaneous air pouch clearly indicating that central formation and action of IL-1beta in this model (91). IL-1ra reduces fever also when microinjected into the anterior hypothalamus, paraventricular hypothalamic nuclei, peri-subfornical organ, subfornical organ or hippocampus (dentate gyrus and CA3 region), but not when administered into the ventromedial hypothalamus, organum vasculosum lamina terminalis (OVLT), CA1 field of the hippocampus, striatum or cortex, indicating a site specific action of endogenous IL-1 in the brain during fever (126).

E. Prostaglandin E2 (PGE2) is considered to be a central mediator of fever. PGE2 is synthesized from arachidonic acid mobilized from membrane lipids by phospholipase A₂ and requires the action of cyclooxygenase (COX) and mPGES (microsomal PGE synthase); cyclooxygenase exists in two forms: constitutive (COX-1) or inducible (COX-2). COX-2 is expressed in brain endothelial cells, perivascular microglia and meningeal macrophages in response to peripheral injection of LPS (150, 151). Peripheral cytokines induce PGE2 release in the brain from blood brain barrier endothelial cells (152, 153). Cytokines and cytokine inducers increase the blood and CSF level of PGE2 as well as PGE2 entry into the third cerebral ventricle as demonstrated by the use of ¹²⁵I-labeled PGE2 (154). Blocking the PGE2 biosynthetic enzymes cyclooxygenase1/2 (COX1/2) (155) or the mPGES (156) are effective measures in blocking fever. Thus, peripheral rise in proinflammatory cytokines can act at the endothelial cells of the blood brain barrier to induce PGE2 release into the brain. Mark and colleagues (157) used primary cultured bovine brain microvessel endothelial cells (BBMECs) as an in vitro model of the blood-brain barrier. They show that TNF treatment of this cellular monolayer causes changes in cytoskeletal structure with formation of actin filament tangles and extracellular gaps, thus altering the permeability. These changes in tight junction permeability were significantly reduced following pretreatment with NS-398 or indomethacin, inhibitors of cyclooxygenase. Moreover, BBMENC monolayers treated with PGE2 showed significant increases in permeability and cytoskeletal structural changes when compared with control monolayers (157).

Blood PGE2 levels are elevated during fever and peripheral inhibition of PGs synthesis block the fever response. Although, peripheral administration of PGE2 did not consistently demonstrate its pyrogenic action, these variations may be due to methodological, issues particularly when the poor water-solubility of PGE2 was not taken into consideration (158). Thus, peripheral PGE2 may also be involved in fever.

It is likely that all these mechanisms contribute to the mechanisms of fever induction by peripheral infection and/or inflammation. Yet, their relative physiological relevance is difficult to evaluate. For example, during bacterial infection, the actions of IL-1 may be similar to that caused by the components of Gram positive or Gram negative bacteria cell walls via stimulation of the Toll receptors TLR2 and 4, including their effects on PGE2 synthesis and release. Finally, all of these mechanisms are bypassed when the brain itself is subjected to trauma with consequent local synthesis of cytokines by activated microglia and to some extent by astroglia and neurons. Seizure activity (24), mechanical trauma (159, 160), ischemia (161-163) and oxidative damage all elevate cytokine levels in the brain with concomitant fever response. Similarly, major damage to the blood brain barrier allows macrophages as well as peripheral cytokine to enter the CNS.

5.2. Actions of cytokines formed in the CNS

IL-1, IL-6 and TNF can be synthesized and are active in the CNS. Most cell types in the brain express cytokine receptors and several studies suggest that low levels of cytokines play a role in neuronal development as trophic factors (164). More importantly cytokine levels can affect neuronal excitability via rapid, non-transcription dependent mechanisms. In experimental models including intracerebroventricular injection of cytokines and transgenic mice overexpressing IL-1ra, the ratio between IL-1beta and IL-1ra was shown to affect memory performance as well as seizure threshold; IL-1beta was indeed demonstrated to be proepileptic and IL-1ra a potent antiepileptic agent (165). It is thus not surprising that elevated IL-1beta levels (without concomitant and fast elevation of IL-1ra levels) are associated with febrile seizure in children (166).

Among the proinflammatory cytokines, IL-6 appears to be the most relevant cytokine with respect to its central role in mediating fever. LPS-induced fever is not fully blocked in IL-1beta null nor in IL-1R null animals but it is not evoked in IL-6 null mice, where it can be rescued by intracerebroventricular injection of IL-6 prior to the peripheral or central injection of LPS in low doses (86). This result suggests that at low and moderate doses (such as those occurring in inflammatory states and infection but not
Cytokines and fever

those prevailing during sepsis) LPS mediated fever requires IL-6 signaling in the brain.

6. EFFECTS OF PYROGENIC CYTOKINES ON THE FIRING RATE OF NEURONS IN THE PREOPTIC AREA / ANTERIOR HYPOTHALAMUS AREA (POA/AH)

Lesion and thermal stimulation studies have demonstrated that the control of body core temperature is primarily mediated by neurons in the anterior area of the hypothalamus. Neurons in these region sense changes in the core and skin and local temperature. Skin thermal afferent signals from both sides of the body converge their action on the preoptic area of the anterior hypothalamus (POAH) (167). POAH warm and cold sensitive neurons using feed forward peripheral afferent input/information, feed-back local temperature information and local hormonal influences (168) coordinate the elicitation of thermoregulatory responses as well as that of the fever responses (for review see 169). This thermoregulatory brain area includes the medial and the lateral part of the preoptic nucleus, the anterior hypothalamus and the nearby regions of the septum and is referred to as the preoptic region (169). Direct thermal stimulation of the brain by warming the blood flow from the carotid produced cutaneous vasodilatation and panting (170) indicating that the CNS can sense and respond to body temperature changes. Similar results were obtained irrigating the third ventricle with warm saline (171). Warming one side of a rat's preoptic area and anterior hypothalamus suppresses shivering on both sides of the body (172). The localization of the preoptic region as the thermosensitive and thermoregulatory area of the brain was demonstrated in experiments on local thermostimulation of the hypothalamus in cats and dogs. Warming of the preoptic area with water perfused thermodes evoked panting, suppressed ongoing shivering and induced ear vasodilatation (173, 174). Cooling of the preoptic area induced heat production by shivering or by increased metabolic activity of brown adipose tissue and increased the circulating levels of thyroxine, catecholamine and glucocorticoids (175-177). In addition to sensing local changes of temperature, the preoptic region also serves as integrator of thermal information from the organism and the environment by receiving afferent sensory input from the body and the skin (178).

In vivo and in vitro (hypothalamic slices) single cell electrophysiological recording experiments in the preoptic region have identified three neuronal cell types based on changes in their firing rate during changes in local hypothalamic temperature: warm sensitive, cold sensitive and temperature insensitive neurons (179-181). Definitions of temperature sensitivity of neurons have been adopted mostly based on changes greater than 0.8 Hz/C in the firing rates of spontaneously active neurons (182) showing a large Q10 value for these neurons. These definitions of temperature sensitivity are subject to discussion because of the arbitrary choice of the Q10 value and also because non-spontaneously active neurons can be “turned into” active and thermosensitive neurons through synaptic actions. Thus thermosensitivity may be a more complex concept and property than the simple definition based on Q10 of spontaneously firing neurons changing their firing rates upon temperature change. Whatever numerical value is accepted to define them, it is clear that the POA and other areas of the CNS contain warm and cold sensitive neurons the activity of which is affected by changes of the temperature. A biochemical measure of these changes is found in turning on the transcription of the early gene c-Fos by warming or cooling these neurons (183, 184). Warm sensitive neurons, accounting for approximately 30% of the neuronal population in the POA, show a significant increase in firing rate when the temperature of the preoptic area is increased from 36ºC to 39ºC. Approximately 5% of the neuronal population in this area (POA) increased their firing rate when the temperature was decreased from 36ºC to 32ºC and are thus often referred to as cold sensitive (although the existence of a population of cold sensitive neurons as such is debated: (181, 185)). Rather, it was proposed that warm sensitive neurons exert synaptic inhibition on the so-called cold sensitive neurons. Warm sensitive neurons decrease their firing rate when temperature drops, thus reducing their synaptic inhibition and allowing an increase of the firing rate of the cold sensitive neurons. The remaining part of the neuronal population of the POA, approximately 65%, shows little or no change in the firing rate when the temperature is either increased or decreased: they are defined as temperature insensitive neurons (181, 185). While the nature of the mechanisms that mediate the integration of the thermal information remain unknown, it has been proposed that these functions are mediated by the neuronal circuitry composed of warm sensitive, cold sensitive and also of temperature insensitive neurons that are innervated by temperature sensitive neurons. Such circuits of temperature sensitive neurons may play a role in temperature homeostasis by comparing excitatory and inhibitory synaptic inputs. Recent electrophysiological analyses have identified some neurons sending axons directly to the spinal cord for thermoregulatory effector control. Included in such thermoregulatory output are midbrain reticulospinal neurons for shivering and premotor neurons in the medulla oblongata for skin vasomotor control. As for the afferent side of the thermoregulatory network, the vagus nerve is recently paid much attention, which would convey signals for peripheral infection to the brain and be responsible for the induction of fever. The vagus nerve may also participate in thermoregulation in febrile conditions. Indeed, some substances such as cholecystokinin and leptin activate the vagus nerve. Although the functional role for this response is still obscure, the vagus may transfer nutritional and/or metabolic signals to the brain, affecting metabolism and body temperature. The thermoregulatory circuits in the POAH respond in a feed forward manner to the afferent signals from skin and core temperatures and use local temperatures for the feed back regulation of the efferent signals through different brain stem structures such as the raphe pallidus, involved in mediating the control of brown adipose tissue (BAT) thermogenesis in rodents (186). In deciding which of the warm and cold sensitive neuron populations in the POA plays a more important role in evoking the thermoregulatory responses such as control of
pathways are activated receive a critical input from the descending heat producing or heat dissipating mechanisms nor heat producing effector responses are controlled by warm-sensitive neurons. These neurons project excitatory efferent signals through the brain stem to control heat loss and send inhibitory efferent signals to control heat production (187). It is important to note that, the “set-point” of the thermoregulatory system can be defined as the mean body temperature (or range of mean body temperatures) at which neither active heat loss mechanisms nor heat producing effector responses are activated. Of course, the hypothalamic structures from which descending heat producing or heat dissipating pathways are activated receive a critical input from the thermosensitive structures. The activity of thermosensitive neurons thus influences the “set-point”, but is not identical with it.

BAT thermogenesis and shivering, Kanosue’s studies point to the greater importance of the warm sensitive neurons as deciding the neuronal output from the POA. Iontophoretic studies on warm sensitive neurons of the preoptic area demonstrate that both heat loss and production responses are controlled by warm-sensitive neurons. These neurons project excitatory efferent signals through the brain stem to control heat loss and send inhibitory efferent signals to control heat production (187). It is important to note that, the “set-point” of the thermoregulatory system can be defined as the mean body temperature (or range of mean body temperatures) at which neither active heat loss mechanisms nor heat producing effector responses are activated. Of course, the hypothalamic structures from which descending heat producing or heat dissipating pathways are activated receive a critical input from the thermosensitive structures. The activity of thermosensitive neurons thus influences the “set-point”, but is not identical with it.

The endogenous pyrogens PGE2, IL-1beta, IL-6 and TNF can directly affect the activity of temperature sensitive neurons suggesting their direct action on the thermoregulatory “set-point”. IL-1beta decreases the activity of most warm-sensitive neurons and increases the activity of most cold-sensitive neurons both in vivo (188) and in vitro (189). Vasilenko and colleagues reported that IL-1beta decreases the firing rate of warm-sensitive neurons in rat brain slices and also reduces their thermosensitivity and shifts the thermal thresholds of activation. (190) The IL-1beta receptor antagonist does not affect neuronal activity but blocks the effect of IL-1beta when administered before the cytokine (92). Similarly, the pyrogenic cytokines TNF-alpha and -beta, IFN-alpha and IL-6 decrease the activity of most warm-sensitive neurons and increase that of most cold-sensitive neurons in rat brain slices (81, 191). Interestingly, when two or more of these pyrogenic cytokines (IL-1beta, TNF and IFN-alpha) are applied to the same neuron, 61% of neurons responded differentially, suggesting the possibility that different cytokines may affect distinct neurons that are functionally responsive to common pyrogenic effectors. The effects of IL-1beta and TNF-beta are blocked by sodium salicylate (a cyclooxygenase inhibitor) suggesting that these effects require local synthesis and release of prostaglandins (188, 189). In contrast, the effects of IFN-alpha on POA neurons are not affected by sodium salicylate but by naloxone (an opioid receptor antagonist). The neuronal effects of IL-6 in POA slices are blocked by either indomethacin (a cyclooxygenase inhibitor) or naloxone suggesting that IFN-alpha acts, in this context, at an allosteric site on the opiate receptor (81).

The above discussed neuronal effects of pyrogenic cytokines IL-1beta, TNF and IL-6 are fast and can therefore most likely represent transcription independent signaling through a respective cytokine receptor system. In the case of the rapid, transcription independent effects of IL-1beta and TNF, the activation of the neutral sphingomyelinase by TNF (p55) receptor and by type 1 IL-1 receptor has been reported (41, 192). The production of ceramide as one of the products of the sphingomyelinase catalytic action is thought to affect the cells by activating protein kinases such as PKC-gamma and the protein tyrosine kinase Src; the downstream effects of the activation of these protein kinases are believed to affect/evoke the rapid changes in firing rates observed upon TNF and IL-beta application, respectively. In support of these assumptions are the findings that cell penetrating ceramide analogs such as C2 ceramide can in vivo mediate fever like rise in temperature when applied intracerebroventricularly (Bartfai et al., unpublished) and that application of C2 ceramide to POA neurons have an like effect (Tabarean et al., unpublished); thus C2 ceramide may IL-1beta mimic the actions of an endogenous ceramide that may act as a second messenger of pyrogenic cytokine action. Thus depending on which neuron is studied and how far we have proceeded in the multiphasic fever response the cellular effects may be mediated by IL-1 at neuronal IL-1type 1 receptors, by PGE2 at EP3 prostanoid receptors or at both receptor types. There is emerging evidence that neurons carrying one or both of these receptors for pyrogens are present in the thermoregulatory area of the POA (Figure 2). The distribution for TNF (p55)

Figure 2. Primary hypothalamic neurons express receptors for pyrogens: the type 1 IL-1 R and the prostanoid receptor EP3. Anterior hypothalamic neuronal cultures containing thermosensitive neurons were established from embryonic E14 mice. After dissection of the anterior hypothalami, cells were plated onto poly-D-lysine coated coverslips and allowed to develop in vitro. Immunocytochemical characterization of these neurons showed expression of neuronal markers such as MAP-2 and the GABA synthetic enzyme; GAD67, as well as molecular markers involved in the mediation of the fever response, such as the type I interleukin receptor (IL-1RI) and the EP3 prostanoid receptor providing a direct anatomical basis for pyrogen mediated effects on the neuronal activity of cells involved in thermoregulation. A percentage of these cells (approximately 10%) respond to temperature changes (not shown).
Cytokines and fever

receptors and for TLR4 type LPS-receptors in the POA is not yet known.

7. CONCLUSION

The initial observation that the leucocytes activating factor, later named IL-1beta, acts as an endogenous pyrogen was followed by the discovery that several other cytokines can contribute to the fever response. The experimental approach to examine their effects on thermoregulation included direct peripheral and central application of cytokines, stimulation of their production, their neutralization with antiserum, soluble receptors or receptor antagonists and the use of transgenic models. This work demonstrated the importance of the rapidly inducible, intercellular messenger molecules cytokines that can mediate the body to brain communication during infection or disease and provided the basis for a deeper understanding of the molecular and cellular mechanisms that regulate the fever response. Although the molecules and mechanisms at play are likely to increase in number as novel cytokines are being discovered, it is possible to summarize some of the most important lessons learned so far. 1. Cytokine action in inducing fever is redundant, i.e. multiple pyrogenic cytokines are induced when fever response is evoked, in fact cytokines are among the most potent inducers of other proinflammatory/pyrogenic cytokines; 2. Both pyrogenic (IL-1alpha/beta, IL-6, CNF etc) and antipyretic (IL-1ra, IL-10 etc) cytokines are induced by most stimuli that cause fever response; 3. Cytokine action on fever can thus be and is, negatively regulated endogenously, explaining the multiphasic nature of the fever responses; 4. Both peripheral and central cytokines can modulate the fever response; 5. A complex network of cytokines exists in the periphery and in the CNS; IL-6 production is modulated by TNF, IFN-gamma and IL-1beta (193), IL-6 receptor is up-regulated in the brain during endotoxemia; IL-6 itself is able to induce the synthesis of its own receptor along cerebral blood vessels and that of the PGE2 synthesizing enzymes COX1 and COX2; 6. PGE2 acting at EP3 prostanoid receptor emerges as an important link between pyrogenic cytokines and fever response; 7. LPS induces its effects via Toll signaling; depending on dose it can activate Toll signaling directly (TLR4) or indirectly via induction of pyrogenic cytokines of which IL-1 acts at IL-1RI-IL-1RacP, through the same Toll signaling pathway that can be initiated by the LPS-LPS binding protein association with TLR4.

These considerations are important in evaluating the complex and multilevel physiological roles of cytokines and their receptors in modulating the fever response and in understanding the molecular, cellular and systemic mechanisms ultimately leading to the up and down regulation of the temperature set-point in the hypothalamus during fever.

8. ACKNOWLEDGEMENTS

The authors thank Margarita Behrens for critical discussion and for her courtesy in providing the pictures primary hypothalamic neurons. Collette Beaton and Janet Molsberry for their help in preparing the manuscript. Supported by NS043501.

9. REFERENCES


Cytokines and fever


35. Gourine A.V., K. Rudolph, J. Tesfaigzi and M.J. Kluger: Role of hypothalamic interleukin-1beta in fever


44. Campbell V. and M.A. Lynch: The role of ceramide in the modulation of intracellular Ca²⁺ levels by interleukin 1 beta in rat cortical synaptosomes. Cytokine 12, 487-490 (2000)


Cytokines and fever


Cytokines and fever


92. Xin L. and C.M. Blatteis: Blockade by interleukin-1 receptor antagonist of IL-1 beta-induced neuronal activity in guinea pig preoptic area slices. *Brain Res* 569, 348-352 (1992)


Cytokines and fever is an adaptor protein in the hToll/IL-1 receptor family signaling pathways. *Mol Cell* 2, 253-258 (1998)


120. Vallieres L. and S. Rivest: Regulation of the genes encoding interleukin-6, its receptor and gp130 in the rat brain in response to the immune activator lipopolysaccharide and the proinflammatory cytokine interleukin-1beta. *J Neurochem* 69, 1668-1683 (1997)


Cytokines and fever


Cytokines and fever


Cytokines and fever


**Key Words** Fever, Cytokine, Interleukin, Pyrogen, Antipyretic, Hypothalamic neuron, Thermoregulation, Core body temperature, Review

**Send correspondence to:** Tamas Bartfai, Harold L. Dorris Neurological Research Center, Department of Neuropsycharmacology, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, California 92037, USA, Tel: 858-784-8404, Fax: 858-784-9099, E-mail: tbartfai@scripps.edu