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CYTOKINES AND FEVER

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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-

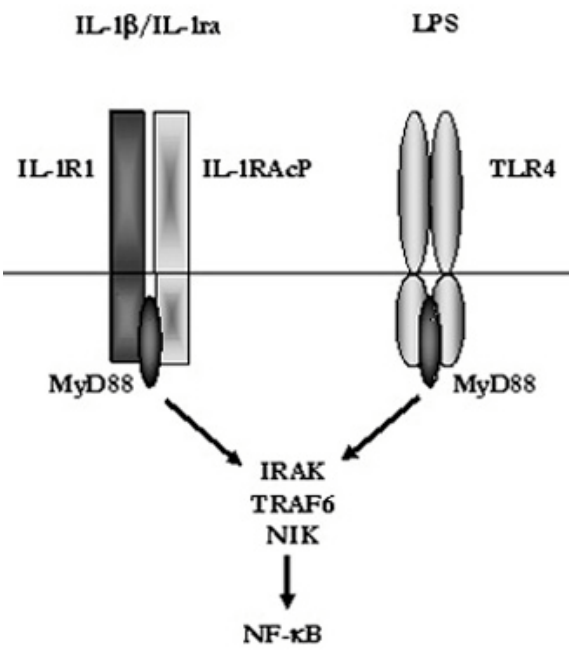

Figure 1. Convergence of IL-1 and LPS signaling. Schematic representation of how IL-1 and LPS action both activate the same intracellular signal transduction pathway. IL-1 beta and the members of IL-1 family of ligands: IL-1alpha/beta and IL-1ra act through the IL-1Type I receptor, a heterodimer of the IL-1RI and its accessory protein IL1RAcP. Binding of agonist, IL-1beta or IL-1alpha induces the recruitment of the cytosolic adaptor protein, MyD88 or its analogs and initiates the activation of the IRAK/TRAF pathway leading to the activation of the transcription factor NF-□B and subsequently to transcriptional stimulation of multiple genes including those coding for COX2 and iNOS and, that are involved in the production of potent inflammatory mediators such as PGE2 and NO and to the auto induction of IL-1beta synthesis, respectively. LPS, bound to the LPS binding protein can activate the same transduction pathway upon stimulation of the TLR4 receptor belonging to the same family of Toll receptors as the IL-1type 1 receptor and utilizing the same IRAK/TRAF pathway leading to the activation of the transcription factor NF-□B with transcriptional consequences as described above (see text for details). Thus LPS can act directly at TLR4 and/or it can induce e.g. IL-1beta synthesis and then evoke similar responses via the IL-1type 1 receptor. Availability of LPS, LPS binding protein and of TLR4 are important in deciding how much of LPS signaling will occur through each branch of this bifurcated signaling leading to the production of the same proinflammatory agents, IL-1beta, PGE2, NO

1 receptor antagonist (IL-1ra) and interleukin 10 (IL-10). IL-1beta and IL-6 are considered to be endogenous pyrogens, while IL-1ra and IL-10 have antipyretic action. TNF has been shown to have both pyrogenic and antipyretic effects depending on the experimental conditions. As these molecules can be synthesized and are active in the immune as well as in the central nervous system, we will often distinguish between their peripheral and central actions.

3. CYTOKINES AS ENDOGENOUS PYROGENIC AND ANTIPYRETIC AGENTS

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 (2).

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.

3.1. Interleukin 1beta and the Interleukin 1system

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)is 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. The type I IL-1 receptor (IL-1RI) is a member of the Toll receptor family (11-15). The heterodimeric IL-1RI 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-κB) (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-1RI, 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-1R1 as well as IL-1RAcP null mutations prevent IL-1beta- induced fever (33, 34). IL-1-induced fever can be blocked by antibodies to IL1 beta injected into the hypothalamus (35) or by pretreatment with cyclooxygenase (COX)1-2 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 modulation 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 $[Ca^{2+}]_i$ in a p42 MAP kinase-dependent manner (44). In rat pinealocytes, ceramide mediates the inhibitory effect of IL-1beta on the L-type Ca^{2+} 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 $I(K_{Ca})$, 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 metalloproteinase 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 TNFR 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, synoviocytes, 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 antiserum (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 bonafide 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 hrIL-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 antiinflammatory 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 andκB 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 antiinflammatory 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

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-κB-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-6been 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, subfornical 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/evoke 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

the anteroventral third ventricle (AV3V)/POA region via the ventral noradrenergic bundle. The norepinephrine released in this site (141) stimulates the local release of PGE_2 (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 mRNAadministered 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 nucleus, 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 A_2 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 ^{125}I-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, BBMEC 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

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 cholecyctokinin 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 thremoregulatory responses such as control of

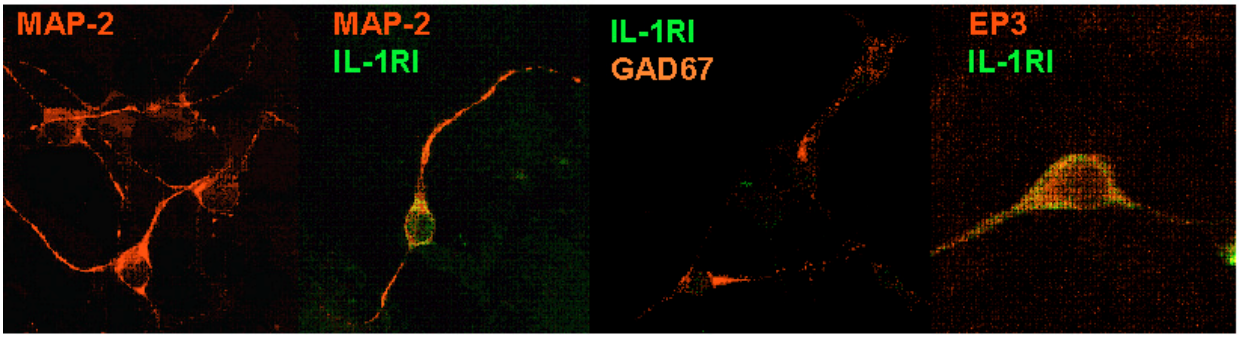

Figure 2. Primary hypothalamic neurons express receptors for pyrogens: the type 1 IL-1 R and the prostanoid receptor EP3. Anterior hypothalamic neuronal cultures containing thermosensitive neurons were established from embryonic E14 mice. After dissection of the anterior hypothalamus, cells were plated 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)

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)

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, CNTF 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.

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9. REFERENCES

1. Dinarello C.A: Cytokines as endogenous pyrogens. *J Infect Dis* 179, Suppl 2, S294-304 (1999)

2. Yoda T., L.I. Crawshaw, K. Yoshida, L. Su, T. Hosono, O. Shido, S. Sakurada, Y. Fukuda and K. Kanosue: Effects of food deprivation on daily changes in body temperature and behavioral thermoregulation in rats. *Am J Physiol - Regul Integr Comp Physiol* 278, R134-139 (2000)

3. Zetterstrom M., A.K. Sundgren-Andersson, P. Ostlund and T. Bartfai: Delineation of the proinflammatory cytokine cascade in fever induction. *Ann NY Acad Sci* 856, 48-52 (1998)

4. Zhang Y.H., J.X. Lin and J. Vilcek: Interleukin-6 induction by tumor necrosis factor and interleukin-1 in human fibroblasts involves activation of a nuclear factor binding to a kappa B-like sequence. *Mol Cell Biol* 10, 3818-3823 (1990)

5. Chow J.C., D.W. Young, D.T. Golenbock, W.J. Christ and F. Gusovsky: Toll-like receptor-4 mediates lipopolysaccharide-induced signal transduction. *J Biol Chem* 274, 10689-10692 (1999)

6. Webb A.C., K.L. Collins, P.E. Auron, R.L. Eddy, H. Nakai, M.G. Byers, L.L. Haley, W.M. Henry and T.B. Shows: Interleukin-1 gene (IL1) assigned to long arm of human chromosome 2. *Lymphokine Res* 5, 77-85 (1986)

7. Nothwang H.G., B. Strahm, D. Denich, M. Kubler, J. Schwabe, J.C. Gingrich, A. Jauch, A. Cox, M.J. Nicklin, D.M. Kurnit and F. Hildebrandt: Molecular cloning of the interleukin-1 gene cluster: construction of an integrated YAC/PAC contig and a partial transcriptional map in the region of chromosome 2q13. *Genomics* 41, 370-378 (1997)

8. Um J.Y., K.S. Moon, K.M. Lee, J.M. Yun, K.H. Cho, B.S. Moon and H.M. Kim: Association of interleukin-1 alpha gene polymorphism with cerebral infarction. *Brain Res Mol Brain Res* 115, 50-54 (2003)

9. Cerretti D.P., L.T. Hollingsworth, C.J. Kozlosky, M.B. Valentine, D.N. Shapiro, S.W. Morris and N. Nelson: Molecular characterization of the gene for human interleukin-1 beta converting enzyme (IL1BC). *Genomics* 20, 468-473 (1994)

10. Dinarello C.A.: Biologic basis for interleukin-1 in disease. *Blood* 87, 2095-2147 (1996)

11. Means T.K., D.T. Golenbock and M.J. Fenton: Structure and function of Toll-like receptor proteins. *Life Sci* 68, 241-258 (2000)

12. Means T.K., D.T. Golenbock and M.J. Fenton: The biology of Toll-like receptors. *Cytokine & Growth Factor Rev* 11, 219-232 (2000)

13. Rock F.L., G. Hardiman, J.C. Timans, R.A. Kastelein and J.F. Bazan: A family of human receptors structurally related to Drosophila Toll. *Proc Natl Acad Sci USA* 95, 588-593 (1998)

14. Dinarello C.A. and N. Savage: Interleukin-1 and its receptor. *Crit Rev Immunol* 9, 1-20 (1989)

15. Heguy A., C.T. Baldari, G. Macchia, J.L. Telford and M. Melli: Amino acids conserved in interleukin-1 receptors (IL-1Rs) and the Drosophila toll protein are essential for IL-1R signal transduction. *J Biol Chem* 267, 2605-2609 (1992)

16. Sims J.E., J.G. Giri and S.K. Dower: The two interleukin-1 receptors play different roles in IL-1 actions. *Clin Immunol Immunopathol* 72, 9-14 (1994)

17. Greenfeder S.A., P. Nunes, L. Kwee, M. Labow, R.A. Chizzonite and G. Ju: Molecular cloning and characterization of a second subunit of the interleukin 1 receptor complex. *J Biol Chem* 270, 13757-13765 (1995)

18. Malinowsky D., Z. Chai, J. Bristulf, A. Simoncsits and T. Bartfai: The type I interleukin-1 receptor mediates fever in the rat as shown by interleukin-1 receptor subtype selective ligands. *Neurosci Lett* 201, 33-36 (1995)

19. Zetterstrom M., J. Lundkvist, D. Malinowsky, G. Eriksson and T. Bartfai: Interleukin-1-mediated febrile responses in mice and interleukin-1 beta activation of NFkappaB in mouse primary astrocytes, involves the interleukin-1 receptor accessory protein. *Eur Cytokine Network* 9, 131-138 (1998)

20. Jefferies C., A. Bowie, G. Brady, E.L. Cooke, X. Li and L.A. O'Neill: Transactivation by the p65 subunit of NF-kappaB in response to interleukin-1 (IL-1) involves MyD88, IL-1 receptor-associated kinase 1, TRAF-6 and Rac1. *Mol Cell Biol* 21, 4544-4552 (2001)

21. Re F., M. Sironi, M. Muzio, C. Matteucci, M. Introna, S. Orlando, G. Penton-Rol, S.K. Dower, J.E. Sims, F. Colotta and A. Mantovani: Inhibition of interleukin-1 responsiveness by type II receptor gene transfer: a surface "receptor" with anti-interleukin-1 function. *J Exp Med* 183, 1841-1850 (1996)

22. Mantovani A., M. Locati, A. Vecchi, S. Sozzani and P. Allavena: Decoy receptors: a strategy to regulate inflammatory cytokines and chemokines. *Trends Immunol* 22, 328-336 (2001)

23. Penton-Rol G., S. Orlando, N. Polentarutti, S. Bernasconi, M. Muzio, M. Introna and A. Mantovani: Bacterial lipopolysaccharide causes rapid shedding, followed by inhibition of mRNA expression, of the IL-1 type II receptor, with concomitant up-regulation of the type I receptor and induction of incompletely spliced transcripts. *J Immunol* 162, 2931-2938 (1999)

24. Virta M., M. Hurme and M. Helminen: Increased plasma levels of pro- and antiinflammatory cytokines in patients with febrile seizures. *Epilepsia* 43, 920-923 (2002)

25. Moldawer L.L., J. Gelin, T. Schersten and K.G. Lundholm: Circulating interleukin 1 and tumor necrosis factor during inflammation. *Am J Physiol* 253, R922-928 (1987)

26. Lyte M.: Regulation of interleukin-1 production in murine macrophages and human monocytes by a normal physiological constituent. *Life Sci* 38, 1163-1170 (1986)

27. Avitsur R., Y. Pollak and R. Yirmiya: Administration of interleukin-1 into the hypothalamic paraventricular nucleus induces febrile and behavioral effects. *Neuroimmunomodulation* 4, 258-265 (1997)

28. Murakami N., Y. Sakata and T. Watanabe: Central action sites of interleukin-1 beta for inducing fever in rabbits. *J Physiol* 428, 299-312 (1990)

29. Nemunaitis J., F.R. Appelbaum, K. Lilleby, W.C. Buhles, C. Rosenfeld, Z.R. Zeigler, R.K. Shadduck, J.W. Singer, W. Meyer and C.D. Buckner: Phase I study of recombinant interleukin-1 beta in patients undergoing autologous bone marrow transplant for acute myelogenous leukemia. *Blood* 83, 3473-3479 (1994)

30. Lundkvist J., A.K. Sundgren-Andersson, S. Tingsborg, P. Ostlund, C. Engfors, K. Alheim, T. Bartfai, K. Iverfeldt and M. Schultzberg: Acute-phase responses in transgenic mice with CNS overexpression of IL-1 receptor antagonist. *Am J Physiol* 276, R644-651 (1999)

31. Opp M.R. and J.M. Krueger: Interleukin 1-receptor antagonist blocks interleukin 1-induced sleep and fever. *Am J Physiol* 260, R453-457 (1991)

32. Luheshi G., A.J. Miller, S. Brouwer, M.J. Dascombe, N.J. Rothwell and S.J. Hopkins: Interleukin-1 receptor antagonist inhibits endotoxin fever and systemic interleukin-6 induction in the rat. *Am J Physiol* 270, E91-95 (1996)

33. Labow M., D. Shuster, M. Zetterstrom, P. Nunes, R. Terry, E.B. Cullinan, T. Bartfai, C. Solorzano, L.L. Moldawer, R. Chizzonite and K.W. McIntyre: Absence of IL-1 signaling and reduced inflammatory response in IL-1 type I receptor-deficient mice. *J Immunol* 159, 2452-2461 (1997)

34. Cullinan E.B., L. Kwee, P. Nunes, D.J. Shuster, G. Ju, K.W. McIntyre, R.A. Chizzonite and M.A. Labow: IL-1 receptor accessory protein is an essential component of the IL-1 receptor. *J Immunol* 161, 5614-5620 (1998)

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

induced by cecal ligation and puncture in rats. *Am J Physiol* 275, R754-761 (1998)

36. Li S., L.R. Ballou, S.G. Morham and C.M. Blatteis: Cyclooxygenase-2 mediates the febrile response of mice to interleukin-1beta. *Brain Res* 910, 163-173 (2001)

37. Ushikubi F., E. Segi, Y. Sugimoto, T. Murata, T. Matsuoka, T. Kobayashi, H. Hizaki, K. Tuboi, M. Katsuyama, A. Ichikawa, T. Tanaka, N. Yoshida and S. Narumiya: Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature* 395, 281-284 (1998)

38. Marsh C.B. and M.D. Wewers: Cytokine-induced interleukin-1 receptor antagonist release in mononuclear phagocytes. *Am J Respir Cell Mol Biol* 10, 521-525 (1994)

39. Foey A.D., S.L. Parry, L.M. Williams, M. Feldmann, B.M. Foxwell and F.M. Brennan: Regulation of monocyte IL-10 synthesis by endogenous IL-1 and TNF-alpha: role of the p38 and p42/44 mitogen-activated protein kinases. *J Immunol* 160, 920-928 (1998)

40. Souza D.G., R. Guabiraba, V. Pinho, A. Bristow, S. Poole and M.M. Teixeira: IL-1-driven endogenous IL-10 production protects against the systemic and local acute inflammatory response following intestinal reperfusion injury. *J Immunol* 170, 4759-4766 (2003)

41. Nalivaeva N.N., E.G. Rybakina, I. Pivanovich, I.A. Kozinets, S.N. Shanin and T. Bartfai: Activation of neutral sphingomyelinase by IL-1beta requires the type 1 interleukin 1 receptor. *Cytokine* 12, 229-232 (2000)

42. Kolesnick R. and D.W. Golde: The sphingomyelin pathway in tumor necrosis factor and interleukin-1 signaling. *Cell* 77, 325-328 (1994)

43. Mathias S., A. Younes, C.C. Kan, I. Orlow, C. Joseph and R.N. Kolesnick: Activation of the sphingomyelin signaling pathway in intact EL4 cells and in a cell-free system by IL-1 beta. *Science* 259, 519-522 (1993)

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)

45. Chik C.L., B. Li, T. Negishi, E. Karpinski and A.K. Ho: Ceramide inhibits L-type calcium channel currents in rat pinealocytes. *Endocrinology* 140, 5682-5690 (1999)

46. Chik C.L., B. Li, E. Karpinski and A.K. Ho: Ceramide inhibits the outward potassium current in rat pinealocytes. *J Neurochem* 79, 339-348 (2001)

47. Boneberg E. and H. T.: Febrile temperatures attenuate IL-1beta release by inhibiting proteolytic processing of the proform and influence Th1/Th2 balance by favoring Th2 cytokines. *J Immunol* 171, 664-668 (2003)

48. Wanebo H.J.: Tumor necrosis factors. *Semin Surgical Oncol* 5, 402-413 (1989)

49. Conti P., R.A. Dempsey, M. Reale, R.C. Barbacane, M.R. Panara, M. Bongrazio and J.W. Mier: Activation of human natural killer cells by lipopolysaccharide and generation of interleukin-1 alpha, beta, tumour necrosis factor and interleukin-6. Effect of IL-1 receptor antagonist. *Immunol* 73, 450-456 (1991)

50. Baumgartner R.A., V.A. Deramo and M.A. Beaven: Constitutive and inducible mechanisms for synthesis and release of cytokines in immune cell lines. *J Immunol* 157, 4087-4093 (1996)

51. Spriggs D.R., S. Deutsch and D.W. Kufe: Genomic structure, induction and production of TNF-alpha. *Immunol Series* 56, 3-34 (1992)

52. Eck M. and S. SR.: The structure of tumor necrosis factor-alpha at 2.6 A resolution. Implications for receptor binding. *J Biol Chem* 264, 17595-17605 (1989)

53. Idriss H.T. and J.H. Naismith: TNF alpha and the TNF receptor superfamily: structure-function relationship(s). *Microscopy Res Tech* 50, 184-195 (2000)

54. Hehlgans T. and D.N. Mannel: The TNF-TNF receptor system. *Biol Chem* 383, 1581-1585 (2002)

55. Chen G. and D.V. Goeddel: TNF-R1 signaling: a beautiful pathway. *Science* 296, 1634-1635 (2002)

56. Himmler A., I. Maurer-Fogy, M. Kronke, P. Scheurich, K. Pfizenmaier, M. Lantz, I. Olsson, R. Hauptmann, C. Stratowa and G.R. Adolf: Molecular cloning and expression of human and rat tumor necrosis factor receptor chain (p60) and its soluble derivative, tumor necrosis factor-binding protein. *DNA Cell Biol* 9, 705-715 (1990)

57. Nakamura H., Y. Seto, S. Motoyoshi, T. Kadokawa and N. Sunahara: Recombinant human tumor necrosis factor causes long-lasting and prostaglandin-mediated fever, with little tolerance, in rabbits. *J Pharmacol Exp Ther* 245, 336-341 (1988)

58. Morimoto A., Y. Sakata, T. Watanabe and N. Murakami: Characteristics of fever and acute-phase response induced in rabbits by IL-1 and TNF. *Am J Physiol* 256, R35-41 (1989)

59. Kawasaki H., M. Moriyama, Y. Ohtani, M. Naitoh, A. Tanaka and H. Nariuchi: Analysis of endotoxin fever in rabbits by using a monoclonal antibody to tumor necrosis factor (cachectin). *Infect Immun* 57, 3131-3135 (1989)

60. Dinarello C.A., J.G. Cannon, S.M. Wolff, H.A. Bernheim, B. Beutler, A. Cerami, I.S. Figari, M.A. Palladino, Jr. and J.V. O'Connor: Tumor necrosis factor (cachectin) is an endogenous pyrogen and induces production of interleukin 1. *J Exp Med* 163, 1433-1450 (1986)

61. Coceani F., J. Lees, J. Mancilla, J. Belizario and C.A. Dinarello: Interleukin-6 and tumor necrosis factor in cerebrospinal fluid: changes during pyrogen fever. *Brain Res* 612, 165-171 (1993)

62. Cooper A.L., S. Brouwer, A.V. Turnbull, G.N. Luheshi, S.J. Hopkins, S.L. Kunkel and N.J. Rothwell: Tumor necrosis factor-alpha and fever after peripheral inflammation in the rat. *Am J Physiol* 267, R1431-1436 (1994)

63. Nagai M., T. Saigusa, Y. Shimada, H. Inagawa, H. Oshima and M. Iriki: Antibody to tumor necrosis factor (TNF) reduces endotoxin fever. *Experientia* 44, 606-607 (1988)

64. Luheshi G.N., A. Stefferl, A.V. Turnbull, M.J. Dascombe, S. Brouwer, S.J. Hopkins and N.J. Rothwell: Febrile response to tissue inflammation involves both peripheral and brain IL-1 and TNF-alpha in the rat. *Am J Physiol* 272, R862-868 (1997)

65. Cartmell T., T. Southgate, G.S. Rees, M.G. Castro, P.R. Lowenstein and G.N. Luheshi: Interleukin-1 mediates a rapid inflammatory response after injection of adenoviral vectors into the brain. *J Neurosci* 19, 1517-1523 (1999)

66. Klir J.J., J.L. McClellan, W. Kozak, Z. Szelenyi, G.H. Wong and M.J. Kluger: Systemic but not central administration of tumor necrosis factor-alpha attenuates LPS-induced fever in rats. *Am J Physiol* 268, R480-486 (1995)

67. Kozak W., C.A. Conn, J.J. Klir, G.H. Wong and M.J. Kluger: TNF soluble receptor and antiserum against TNF enhance lipopolysaccharide fever in mice. *Am. J Physiol* 269, R23-29 (1995)

68. Leon L.R., A.A. White and M.J. Kluger: Role of IL-6 and TNF in thermoregulation and survival during sepsis in mice. *Am J Physiol* 275, R269-277 (1998)

69. Roth J., T. Hubschle, U. Pehl, G. Ross and R. Gerstberger: Influence of systemic treatment with cyclooxygenase inhibitors on lipopolysaccharide-induced fever and circulating levels of cytokines and cortisol in guinea-pigs. *Pflugers Archiv - Eur J Physiol* 443, 411-417 (2002)

70. Romanovsky A.A., C.T. Simons, M. Szekely and V.A. Kulchitsky: The vagus nerve in the thermoregulatory response to systemic inflammation. *Am J Physiol* 273, R407-413 (1997)

71. Romanovsky A.A. and M. Szekely: Fever and hypothermia: two adaptive thermoregulatory responses to systemic inflammation. *Medical Hypotheses* 50, 219-226 (1998)

72. Dogan M.D., H. Ataoglu and E.S. Akarsu: Nimesulide and diclofenac inhibit lipopolysaccharide-induced hypothermia and tumour necrosis factor-alpha elevation in rats. *Fundam Clin Pharmacol* 16, 303-309 (2002)

73. Wang J., T. Ando and A.J. Dunn: Effect of homologous interleukin-1, interleukin-6 and tumor necrosis factor-alpha on the core body temperature of mice. *Neuroimmunomodulation* 4, 230-236 (1997)

74. Alheim K., Z. Chai, G. Fantuzzi, H. Hasanvan, D. Malinowsky, E. Di Santo, P. Ghezzi, C.A. Dinarello and T. Bartfai: Hyperresponsive febrile reactions to interleukin (IL) 1alpha and IL-1beta and altered brain cytokine mRNA and serum cytokine levels, in IL-1beta-deficient mice. *Proc Nat Acad Sci USA* 94, 2681-2686 (1997)

75. Ferguson-Smith A.C., Y.F. Chen, M.S. Newman, L.T. May, P.B. Sehgal and F.H. Ruddle: Regional localization of the interferon-beta 2/B-cell stimulatory factor 2/hepatocyte stimulating factor gene to human chromosome 7, 15-21. *Genomics* 2, 203-208 (1988)

76. Maimone D., C. Cioni, S. Rosa, G. Macchia, F. Aloisi and P. Annunziata: Norepinephrine and vasoactive intestinal peptide induce IL-6 secretion by astrocytes: synergism with IL-1 beta and TNF alpha. *J Neuroimmunol* 47, 73-81 (1993)

77. Frei K., U.V. Malipiero, T.P. Leist, R.M. Zinkernagel, M.E. Schwab and A. Fontana: On the cellular source and function of interleukin 6 produced in the central nervous system in viral diseases. *Eur J Immunol* 19, 689-694 (1989)

78. Juttler E., V. Tarabin and M. Schwaninger: Interleukin-6 (IL-6): a possible neuromodulator induced by neuronal activity. *Neuroscientist* 8, 268-275 (2002)

79. Kishimoto T., M. Hibi, M. Murakami, M. Narazaki, M. Saito and T. Taga: The molecular biology of interleukin 6 and its receptor. *Ciba Foundation Symposium* 167, 5-16, discussion 16-23 (1992)

80. Taga T.: The interleukin-6 signal transducer, gp130, functioning in immune, hematopoietic and neural systems. *Nippon Rinsho - Jap J Clin Med* 50, 1802-1810 (1992)

81. Xin L. and C.M. Blatteis: Hypothalamic neuronal responses to interleukin-6 in tissue slices: effects of indomethacin and naloxone. *Brain Res Bull* 29, 27-35 (1992)

82. Ueno Y., N. Takano, H. Kanegane, T. Yokoi, A. Yachie, T. Miyawaki and N. Taniguchi: The acute phase nature of interleukin 6: studies in Kawasaki disease and other febrile illnesses. *Clin Exp Immunol* 76, 337-342 (1989)

83. Miller A.J., G.N. Luheshi, N.J. Rothwell and S.J. Hopkins: Local cytokine induction by LPS in the rat air pouch and its relationship to the febrile response. *Am J Physiol* 272, R857-861 (1997)

84. Cartmell T., S. Poole, A.V. Turnbull, N.J. Rothwell and G.N. Luheshi: Circulating interleukin-6 mediates the febrile response to localised inflammation in rats.[comment]. *J Physiol* 526, Pt 3, 653-661 (2000)

85. Klir J.J., J.L. McClellan and M.J. Kluger: Interleukin-1 beta causes the increase in anterior hypothalamic interleukin-6 during LPS-induced fever in rats. *Am J Physiol* 266, R1845-1848 (1994)

86. Chai Z., S. Gatti, C. Toniatti, V. Poli and T. Bartfai: Interleukin (IL)-6 gene expression in the central nervous system is necessary for fever response to lipopolysaccharide or IL-1 beta: a study on IL-6-deficient mice. *J Exp Med* 183, 311-316 (1996)

87. LeMay L.G., A.J. Vander and M.J. Kluger: Role of interleukin 6 in fever in rats. *Am J Physiol* 258, R798-803 (1990)

88. Cao C., K. Matsumura, N. Shirakawa, M. Maeda, I. Jikihara, S. Kobayashi and Y. Watanabe: Pyrogenic cytokines injected into the rat cerebral ventricle induce cyclooxygenase-2 in brain endothelial cells and also upregulate their receptors. *Eur J Neurosci* 13, 1781-1790 (2001)

89. Hannum C.H., C.J. Wilcox, W.P. Arend, F.G. Joslin, D.J. Dripps, P.L. Heimdal, L.G. Armes, A. Sommer, S.P. Eisenberg and R.C. Thompson: Interleukin-1 receptor antagonist activity of a human interleukin-1 inhibitor. *Nature* 343, 336-340 (1990)

90. Cartmell T., G.N. Luheshi, S.J. Hopkins, N.J. Rothwell and S. Poole: Role of endogenous interleukin-1 receptor antagonist in regulating fever induced by localised inflammation in the rat. *J Physiol* 531, 171-180 (2001)

91. Miller A.J., S.J. Hopkins and G.N. Luheshi: Sites of action of IL-1 in the development of fever and cytokine responses to tissue inflammation in the rat. *Br J Pharmacol* 120, 1274-1279 (1997)

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)

93. de Waal Malefyt R., H. Yssel, M.G. Roncarolo, H. Spits and J.E. de Vries: Interleukin-10. *Curr Op Immunol* 4, 314-320 (1992)

94. Platzer C., C. Meisel, K. Vogt, M. Platzer and H.D. Volk: Up-regulation of monocytic IL-10 by tumor necrosis factor-alpha and cAMP elevating drugs. *Int Immunol* 7, 517-523 (1995)

95. Platzer C., W. Docke, H. Volk and S. Prosch: Catecholamines trigger IL-10 release in acute systemic stress reaction by direct stimulation of its promoter/enhancer activity in monocytic cells. *J Neuroimmunol* 105, 31-38 (2000)

96. Kotenko S.V. and S. Pestka: Jak-Stat signal transduction pathway through the eyes of cytokine class II receptor complexes. *Oncogene* 19, 2557-2565 (2000)

97. Ward P.A. and A.B. Lentsch: Endogenous regulation of the acute inflammatory response. *Mol Cell Biochem* 234-235, 225-228 (2002)

98. Schottelius A.J., M.W. Mayo, R.B. Sartor and A.S. Baldwin, Jr.: Interleukin-10 signaling blocks inhibitor of kappaB kinase activity and nuclear factor kappaB DNA binding. *J Biol Chem* 274, 31868-31874 (1999)

99. Leon L.R., W. Kozak, K. Rudolph and M.J. Kluger: An antipyretic role for interleukin-10 in LPS fever in mice. *Am J Physiol* 276, R81-89 (1999)

100. Nava F., G. Calapai, G. Facciola, S. Cuzzocrea, M.C. Marciano, A. De Sarro and A.P. Caputi: Effects of interleukin-10 on water intake, locomotory activity and rectal temperature in rat treated with endotoxin. *Int J Immunopharmacol* 19, 31-38 (1997)

101. Cartmell T., C. Ball, A.F. Bristow, D. Mitchell and S. Poole: Endogenous interleukin-10 is required for the defervescence of fever evoked by local lipopolysaccharide-induced and Staphylococcus aureus-induced inflammation in rats. *J Physiol* 549 (Pt 2), 653-664 (2003)

102. Lien E., T.K. Means, H. Heine, A. Yoshimura, S. Kusumoto, K. Fukase, M.J. Fenton, M. Oikawa, N. Qureshi, B. Monks, R.W. Finberg, R.R. Ingalls and D.T. Golenbock: Toll-like receptor 4 imparts ligand-specific recognition of bacterial lipopolysaccharide. *J Clin Invest* 105, 497-504 (2000)

103. Fantuzzi G., H. Zheng, R. Faggioni, F. Benigni, P. Ghezzi, J.D. Sipe, A.R. Shaw and C.A. Dinarello: Effect of endotoxin in IL-1 beta-deficient mice. *J Immunol* 157, 291-296 (1996)

104. Leon L.R., W. Kozak, J. Peschon and M.J. Kluger: Exacerbated febrile responses to LPS, but not turpentine, in TNF double receptor-knockout mice. *Am J Physiol* 272, R563-569 (1997)

105. Poltorak A., X. He, I. Smirnova, M.Y. Liu, C.V. Huffel, X. Du, D. Birdwell, E. Alejos, M. Silva, C. Galanos, M. Freudenberg, P. Ricciardi-Castagnoli, B. Layton and B. Beutler: Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutations in Tlr4 gene. *Science* 282, 2085-2088 (1998)

106. Takeuchi O., K. Hoshino, T. Kawai, H. Sanjo, H. Takada, T. Ogawa, K. Takeda and S. Akira: Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity* 11, 443-451 (1999)

107. Kawai T., O. Adachi, T. Ogawa, K. Takeda and S. Akira: Unresponsiveness of MyD88-deficient mice to endotoxin. *Immunity* 11, 115-122 (1999)

108. Medzhitov R., P. Preston-Hurlburt, E. Kopp, A. Stadlen, C. Chen, S. Ghosh and C.A. Janeway, Jr.: MyD88

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

109. Blatteis C.M.: The afferent signalling of fever.[comment]. *J Physiol* 526, Pt 3, 470 (2000)

110. Netea M.G., B.J. Kullberg and J.W. Van der Meer: Circulating cytokines as mediators of fever. *Clin Infect Dis* 31, Suppl 5, S178-184 (2000)

111. Banks W.A., L. Ortiz, S.R. Plotkin and A.J. Kastin: Human interleukin (IL) 1 alpha, murine IL-1 alpha and murine IL-1 beta are transported from blood to brain in the mouse by a shared saturable mechanism. *J Pharmacol Exp Ther* 259, 988-996 (1991)

112. Banks W.A. and A.J. Kastin: Blood to brain transport of interleukin links the immune and central nervous systems. *Life Sci* 48, PL117-121 (1991)

113. Banks W.A., S.R. Plotkin and A.J. Kastin: Permeability of the blood-brain barrier to soluble cytokine receptors. *Neuroimmunomodulation* 2, 161-165 (1995)

114. McLay R.N., A.J. Kastin and J.E. Zadina: Passage of interleukin-1-beta across the blood-brain barrier is reduced in aged mice: a possible mechanism for diminished fever in aging. *Neuroimmunomodulation* 8, 148-153 (2000)

115. Banks W.A., A.J. Kastin and E.G. Gutierrez: Penetration of interleukin-6 across the murine blood-brain barrier. *Neurosci Lett* 179, 53-56 (1994)

116. Gutierrez E.G., W.A. Banks and A.J. Kastin: Blood-borne interleukin-1 receptor antagonist crosses the blood-brain barrier. *J Neuroimmunol* 55, 153-160 (1994)

117. Kastin A.J., V. Akerstrom and W. Pan: Interleukin-10 as a CNS therapeutic: the obstacle of the blood-brain/blood-spinal cord barrier. *Brain Res Mol Brain Res* 114, 168-171 (2003)

118. Pan W. and A.J. Kastin: TNFalpha transport across the blood-brain barrier is abolished in receptor knockout mice. *Exp Neurol* 174, 193-200 (2002)

119. Osburg B., C. Peiser, D. Domling, L. Schomburg, Y.T. Ko, K. Voigt and U. Bickel: Effect of endotoxin on expression of TNF receptors and transport of TNF-alpha at the blood-brain barrier of the rat. *Am J Physiol - Endocrinol Metab* 283, E899-908 (2002)

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)

121. Ericsson A., C. Liu, R.P. Hart and P.E. Sawchenko: Type 1 interleukin-1 receptor in the rat brain: distribution, regulation and relationship to sites of IL-1-induced cellular activation. *J Comp Neurol* 361, 681-698 (1995)

122. Blatteis C.M.: Role of the OVLT in the febrile response to circulating pyrogens. *Prog Brain Res* 91, 409-412 (1992)

123. Hori T., T. Kiyohara, T. Nakashima, K. Mizuno, H. Muratani and T. Katafuchi: Effects of temperature and neuroactive substances on hypothalamic neurones in vitro: possible implications for the induction of fever. *Physiol Res* 41, 77-81 (1992)

124. Lin M.T. and J.H. Lin: Involvement of tyrosine kinase in the pyrogenic fever exerted by NOS pathways in organum vasculosum laminae terminalis. *Neuropharmacology* 39, 347-352 (2000)

125. Matsumura K., Y. Watanabe, H. Onoe and O. Hayaishi: High density of prostaglandin E2 binding sites in the anterior wall of the 3rd ventricle: a possible site of its hyperthermic action. *Brain Res* 533, 147-151 (1990)

126. Cartmell T., G.N. Luheshi and N.J. Rothwell: Brain sites of action of endogenous interleukin-1 in the febrile response to localized inflammation in the rat. *J Physiol* 518, 585-594 (1999)

127. Romanovsky A.A., A.I. Ivanov and M. Szekely: Neural route of pyrogen signaling to the brain. *Clin Infect Dis* 31, Suppl 5, S162-167 (2000)

128. Maier S.F., L.E. Goehler, M. Fleshner and L.R. Watkins: The role of the vagus nerve in cytokine-to-brain communication. *Ann NY Acad Sci* 840, 289-300 (1998)

129. Watkins L.R., S.F. Maier and L.E. Goehler: Cytokine-to-brain communication: a review & analysis of alternative mechanisms. *Life Sci* 57, 1011-1026 (1995)

130. Szekely M., M. Balasko and A.A. Romanovsky: Peripheral neural inputs. Their role in fever development. *Ann. NY Acad Sci* 813, 427-434 (1997)

131. Goehler L.E., J.K. Relton, D. Dripps, R. Kiechle, N. Tartaglia, S.F. Maier and L.R. Watkins: Vagal paraganglia bind biotinylated interleukin-1 receptor antagonist: a possible mechanism for immune-to-brain communication. *Brain Res Bull* 43, 357-364 (1997)

132. Watkins L.R., L.E. Goehler, J.K. Relton, N. Tartaglia, L. Silbert, D. Martin and S.F. Maier: Blockade of interleukin-1 induced hyperthermia by subdiaphragmatic vagotomy: evidence for vagal mediation of immune-brain communication. *Neurosci Lett* 183, 27-31 (1995)

133. Hansen M.K. and J.M. Krueger: Subdiaphragmatic vagotomy blocks the sleep- and fever-promoting effects of interleukin-1beta. *Am J Physiol* 273, R1246-1253 (1997)

134. Roth J. and G.E. De Souza: Fever induction pathways: evidence from responses to systemic or local cytokine formation. *Braz J Med Biol Res* 34, 301-314 (2001)

135. Sehic E. and C.M. Blatteis: Blockade of lipopolysaccharide-induced fever by subdiaphragmatic vagotomy in guinea pigs. *Brain Res* 726, 160-166 (1996)

136. Romanovsky A.A., C.T. Simons and V.A. Kulchitsky: "Biphasic" fevers often consist of more than two phases. *Am J Physiol* 275, R323-331 (1998)

137. Hansen M.K., K.A. O'Connor, L.E. Goehler, L.R. Watkins and S.F. Maier: The contribution of the vagus nerve in interleukin-1beta-induced fever is dependent on dose. *Am J Physiol - Regul Integr Comp Physiol* 280, R929-934 (2001)

138. Niijima A.: The afferent discharges from sensors for interleukin 1 beta in the hepatoportal system in the anesthetized rat. *J Auton Nervous System* 61, 287-291 (1996)

139. Ek M., M. Kurosawa, T. Lundeberg and A. Ericsson: Activation of vagal afferents after intravenous injection of interleukin-1beta: role of endogenous prostaglandins. *J Neurosci* 18, 9471-9479 (1998)

140. Ek M., C. Arias, P. Sawchenko and A. Ericsson-Dahlstrand: Distribution of the EP3 prostaglandin E(2) receptor subtype in the rat brain: relationship to sites of interleukin-1-induced cellular responsiveness. *J Comp Neurol* 428, 5-20 (2000)

141. Linthorst A.C., C. Flachskamm, F. Holsboer and J.M. Reul: Intraperitoneal administration of bacterial endotoxin enhances noradrenergic neurotransmission in the rat preoptic area: relationship with body temperature and hypothalamic--pituitary--adrenocortical axis activity. *Eur J Neurosci* 7, 2418-2430 (1995)

142. Sehic E., A.L. Ungar and C.M. Blatteis: Interaction between norepinephrine and prostaglandin E2 in the preoptic area of guinea pigs. *Am J Physiol* 271, R528-536 (1996)

143. Luheshi G.N., R.M. Bluthe, D. Rushforth, N. Mulcahy, J.P. Konsman, M. Goldbach and R. Dantzer: Vagotomy attenuates the behavioural but not the pyrogenic effects of interleukin-1 in rats. *Auton. Neurosci-Basic Clin* 85, 127-132 (2000)

144. Konsman J.P., G.N. Luheshi, R.M. Bluthe and R. Dantzer: The vagus nerve mediates behavioural depression, but not fever, in response to peripheral immune signals; a functional anatomical analysis. *Eur J Neurosci* 12, 4434-4446 (2000)

145. Tilders F.J., R.H. DeRijk, A.M. Van Dam, V.A. Vincent, K. Schotanus and J.H. Persoons: Activation of the hypothalamus-pituitary-adrenal axis by bacterial endotoxins: routes and intermediate signals. *Psychoneuroendocrinology* 19, 209-232 (1994)

146. Matsumura K., C. Cao, M. Ozaki, H. Morii, K. Nakadate and Y. Watanabe: Brain endothelial cells express cyclooxygenase-2 during lipopolysaccharide-induced fever: light and electron microscopic immunocytochemical studies. *J Neurosci* 18, 6279-6289 (1998)

147. Hashimoto M., Y. Ishikawa, S. Yokota, F. Goto, T. Bando, Y. Sakakibara and M. Iriki: Action site of circulating interleukin-1 on the rabbit brain. *Brain Res* 540, 217-223 (1991)

148. Quan N., E.L. Stern, M.B. Whiteside and M. Herkenham: Induction of proinflammatory cytokine mRNAs in the brain after peripheral injection of subseptic doses of lipopolysaccharide in the rat. *J Neuroimmunol* 93, 72-80 (1999)

149. Breder C.D., C. Hazuka, T. Ghayur, C. Klug, M. Huginin, K. Yasuda, M. Teng and C.B. Saper: Regional induction of tumor necrosis factor alpha expression in the mouse brain after systemic lipopolysaccharide administration. *Proc Nat Acad Sci US A* 91, 11393-11397 (1994)

150. Cao C., K. Matsumura, K. Yamagata and Y. Watanabe: Induction by lipopolysaccharide of cyclooxygenase-2 mRNA in rat brain; its possible role in the febrile response. *Brain Res* 697, 187-196 (1995)

151. Elmquist J.K., C.D. Breder, J.E. Sherin, T.E. Scammell, W.F. Hickey, D. Dewitt and C.B. Saper: Intravenous lipopolysaccharide induces cyclooxygenase 2-like immunoreactivity in rat brain perivascular microglia and meningeal macrophages. *J Comp Neurol* 381, 119-129 (1997)

152. Engblom D., M. Ek, S. Saha, A. Ericsson-Dahlstrand, P.J. Jakobsson and A. Blomqvist: Prostaglandins as inflammatory messengers across the blood-brain barrier. *J Mol Med* 80, 5-15 (2002)

153. Stitt J.T.: Passage of immunomodulators across the blood-brain barrier. *Yale J Biol Med* 63, 121-131 (1990)

154. Davidson J., H.T. Abul, A.S. Milton and D. Rotondo: Cytokines and cytokine inducers stimulate prostaglandin E2 entry into the brain. *Pflugers Archiv - Eur J Physiol* 442, 526-533 (2001)

155. Cao C., K. Matsumura and Y. Watanabe: Induction of cyclooxygenase-2 in the brain by cytokines. *Ann NY Acad Sci* 813, 307-309 (1997)

156. Stichtenoth D.O., S. Thoren, H. Bian, M. Peters-Golden, P.J. Jakobsson and L.J. Crofford: Microsomal prostaglandin E synthase is regulated by proinflammatory cytokines and glucocorticoids in primary rheumatoid synovial cells. *J Immunol* 167, 469-474 (2001)

157. Mark K.S., W.J. Trickler and D.W. Miller: Tumor necrosis factor-alpha induces cyclooxygenase-2 expression and prostaglandin release in brain microvessel endothelial cells. *J Pharmacol Exp Ther* 297, 1051-1058 (2001)

158. Romanovsky A.A., A.I. Ivanov and E.K. Karman: Blood-borne, albumin-bound prostaglandin E2 may be involved in fever. *Am J Physiol* 276, R1840-1844 (1999)

159. Lau L.T. and A.C. Yu: Astrocytes produce and release interleukin-1, interleukin-6, tumor necrosis factor alpha and interferon-gamma following traumatic and metabolic injury. *J Neurotrauma* 18, 351-359 (2001)

160. Thompson H.J., N.C. Tkacs, K.E. Saatman, R. Raghupathi and T.K. McIntosh: Hyperthermia following traumatic brain injury: a critical evaluation. *Neurobiol Dis* 12, 163-173 (2003)

161. Patel H.C., H. Boutin and S.M. Allan: Interleukin-1 in the brain: mechanisms of action in acute neurodegeneration. *Ann NY Acad Sci* 992, 39-47 (2003)

162. Dziewulska D. and M.J. Mossakowski: Cellular expression of tumor necrosis factor a and its receptors in human ischemic stroke. *Clini Neuropathol* 22, 35-40 (2003)

163. De Simoni M.G., P. Milia, M. Barba, A. De Luigi, L. Parnetti and V. Gallai: The inflammatory response in cerebral ischemia: focus on cytokines in stroke patients. *Clin Exp Hypertension (NY)* 24, 535-542 (2002)

164. Kahn M.A., J.A. Ellison, G.J. Speight and J. de Vellis: CNTF regulation of astrogliosis and the activation of microglia in the developing rat central nervous system. *Brain Res* 685, 55-67 (1995)

165. Vezzani A., D. Moneta, M. Conti, C. Richichi, T. Ravizza, A. De Luigi, M.G. De Simoni, G. Sperk, S. Andell-Jonsson, J. Lundkvist, K. Iverfeldt and T. Bartfai: Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. *Proc Nat Acad Sci USA* 97, 11534-11539 (2000)

166. Haspolat S., E. Mihci, M. Coskun, S. Gumuslu, T. Ozbenm and O. Yegin: Interleukin-1beta, tumor necrosis factor-alpha and nitrite levels in febrile seizures. *J Child Neurol* 17, 749-751 (2002)

167. Kanosue K., K. Niwa, P.D. Andrew, H. Yasuda, M. Yanase, H. Tanaka and K. Matsumura: Lateral distribution of hypothalamic signals controlling thermoregulatory vasomotor activity and shivering in rats. *Am J Physiol* 260, R486-493 (1991)

168. Tsai C.L., K. Kanosue and K. Matsumura: Effects of estradiol treatment on responses of rat preoptic warm sensitive neurons to progesterone *in vitro*. *Neurosci Lett* 136, 23-26 (1992)

169. Boulant J.A.: Role of the preoptic-anterior hypothalamus in thermoregulation and fever. *Clin Infect Dis* 31, Suppl 5, S157-161 (2000)

170. Moorhouse V.: Effect of increased temperature of the carotid blood. *Am J Physiol* 223-234 (1911)

171. Hammouda M.: The central and reflex mechanism of panting. *J Physiol* 77, (1933)

172. Kanosue K., Y.H. Zhang, M. Yanase-Fujiwara and T. Hosono: Hypothalamic network for thermoregulatory shivering. *Am J Physiol* 267, R275-282 (1994)

173. Magoun H., Harrison F, Broebeck JR, Ranson SW.: Activation of heat loss mechanisms by local heating of the brain. *J Neurophysiol* 1, 101-114 (1938)

174. Hemingway H., Rasmussen T, Wikoff H, Rasmussen AT.: Effects of heating hypothalamus of dogs by diathermy. *J Neurophysiol* 329-338 (1940)

175. Jacobson F.H. and R.D. Squires: Thermoregulatory responses of the cat to preoptic and environmental temperatures. *Am J Physiol* 218, 1575-1582 (1970)

176. Boulant J.A. and R.R. Gonzalez: The effect of skin temperature on the hypothalamic control of heat loss and heat production. *Brain Res* 120, 367-372 (1977)

177. Imai-Matsumura K. and T. Nakayama: The central efferent mechanism of brown adipose tissue thermogenesis induced by preoptic cooling. *Can J Physiol Pharmacol* 65, 1299-1303 (1987)

178. Boulant J.A. and J.D. Hardy: The effect of spinal and skin temperatures on the firing rate and thermosensitivity of preoptic neurones. *J Physiol* 240, 639-660 (1974)

179. Nakayama T., Hammel, H.T., Hardy, J.D., Eisenman, J.S.: Thermal stimulation of electrical activity of single units of the preoptic region. *Am J Physiol* 204, 1122-1126 (1963)

180. Boulant J.A., Hypothalamic Control of thermoregulation: neurophysiological basis. In: *Handbook of the hypothalamus*. Ed: Morgane P.J., Panksepp J, Marcel dekker: N.Y. 3A, 1-82 (1980)

181. Boulant J.A., Hypothalamic neurons regulating body temperature. In: *APS handbook of physiology, section 4: environmental physiology*, Ed: Fregly MJ, Blatteis CM, Oxford Press: N.Y. 105-126 (1996)

182. Boulant J.A., A.R. Chow and J.D. Griffin: Determinants of hypothalamic neuronal thermosensitivity. *Ann NY Acad Sci* 813, 133-138 (1997)

183. Yoshida K., M. Maruyama, T. Hosono, K. Nagashima, Y. Fukuda, R. Gerstberger and K. Kanosue: Fos expression induced by warming the preoptic area in rats. *Brain Res* 933, 109-117 (2002)

184. Maruyama M N.M., Konishi M, Takashige Y, Nagashima K, Kiyohara T, Kanosue K.: Brain regions expressing Fos during thermoregulatory behavior in rats. *Am J Physiol Regul Integr Comp Physiol* 285, R1116-1123 (2003)

185. Boulant J.A.: Hypothalamic neurons. Mechanisms of sensitivity to temperature. *Ann NY Acad Sci* 856, 108-115 (1998)

186. Taniguchi A., X.M. Chen, K. Nagashima, M. Tanaka and K. Kanosue: Involvement of the raphe pallidus in the suppressive effect of preoptic warming on non-shivering thermogenesis in rats. *Brain Res* 966, 103-109 (2003)

187. Nagashima K., S. Nakai, M. Tanaka and K. Kanosue: Neuronal circuitries involved in thermoregulation. *Auton Neurosci-Basic Clin* 85, 18-25 (2000)

188. Hori T., M. Shibata, T. Nakashima, M. Yamasaki, A. Asami, T. Asami and H. Koga: Effects of interleukin-1 and arachidonate on the preoptic and anterior hypothalamic neurons. *Brain Res Bull* 20, 75-82 (1988)

189. Nakashima T., T. Hori, T. Mori, K. Kuriyama and K. Mizuno: Recombinant human interleukin-1 beta alters the activity of preoptic thermosensitive neurons in vitro. *Brain Res Bull* 23, 209-213 (1989)

190. Vasilenko V.Y., T.A. Petruchuk, V.N. Gourine and F.K. Pierau: Interleukin-1beta reduces temperature sensitivity but elevates thermal thresholds in different populations of warm-sensitive hypothalamic neurons in rat brain slices. *Neurosci Lett* 292, 207-210 (2000)

191. Nakashima T., T. Kiyohara and T. Hori: Tumor necrosis factor-beta specifically inhibits the activity of preoptic warm-sensitive neurons in tissue slices. *Neurosci Lett* 128, 97-100 (1991)

192. Kuno K. and K. Matsushima: The IL-1 receptor signaling pathway. *J Leukocyte Biol* 56, 542-547 (1994)

193. Norris J.G., L.P. Tang, S.M. Sparacio and E.N. Benveniste: Signal transduction pathways mediating astrocyte IL-6 induction by IL-1 beta and tumor necrosis factor-alpha. *J Immunol* 152, 841-850 (1994)

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