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SIGNALING THE BRAIN IN SYSTEMIC INFLAMMATION: ROLE OF SENSORY CIRCUMVENTRICULAR ORGANS

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1. ABSTRACT

The sensory circumventricular organs (CVOs) are specialized brain regions that lack a tight blood-brain barrier. A role for these brain structures in signaling the brain during systemic inflammation is based on the following sets of observations. In spite of some conflicting data from literature, lesions of CVOs have been shown to block several components of brain controlled illness responses (i.e. fever or neuroendocrine modifications). Receptors for inflammatory cytokines and for bacterial fragments are constitutively expressed in cells within the sensory CVOs. The expression of most of these receptors is upregulated under conditions of systemic inflammation. Cellular responses in theses brain areas can be recorded and documented after stimulation of these respective receptors. Such responses include changes in electrical activity of neurons, induction of transcription factors leading to modifications in gene expression during inflammation and to a localized release of secondary signal molecules. These molecules may influence or even gain access to neural structures inside the blood-brain barrier, which can normally not directly be reached by circulating cytokines or bacterial fragments.

2. INTRODUCTION

In 1958 circumventricular organs (CVOs) were described and characterized morphologically as brain structures having cells in contact with the cerebroventricular system, a dense vascularization and an absence of a blood-brain barrier (1). Structural similarities are found in a CVO-subgroup named sensory CVOs: the vascular organ of the lamina terminalis (OVLT), the subfornical organ (SFO) and the area postrema (AP). These CVOs possess capillaries with a fenestrated endothelium surrounded by perivascular spaces. They are separated from the ventricles by ependymal cells. The parenchyma of these organs is composed of glial cells and of neuronal elements including cell somata, dendritic or axonal processes and nerve terminals (2). These properties suggest that the sensory CVOs share most functional capacities with other areas of the central nervous system (CNS) with one exception: due to the lack of a blood-brain barrier the cells within the sensory CVOs are directly exposed to circulating signal molecules and can thus act as sensors for chemical messengers which are transported by the bloodstream. The OVLT and the SFO are located within the anterior wall of the third ventricle, the so-called lamina terminalis. The AP is a component of the dorsal vagal complex, a major viscerosensory and autonomic center of the medulla oblongata. The locations of these sensory CVOs are schematically shown on a diagram of a mid-sagittal section through a rat brain (Figure 1).

Traditionally, the sensory CVOs are regarded as cerebral structures which are involved in the maintenance of body fluid and cardiovascular homeostasis (3, 4). OVLT, SFO and AP are able to detect circulating hormones which play a role in these functional circuits due to the expression of binding sites for angiotensin II, atrial natriuretic factor, noradrenaline or endothelin (5, 6, 7). The neuronal connections with and the adjacent location to important hypothalamic control centers for vital homeostatic functions infer a critical role of the sensory CVOs in the transfer of blood-borne signals to the hypothalamus.

In 1948, Beeson (8) proposed the hypothesis that fever is induced by an endogenous molecule, a pyrogen which is produced by activated leukocytes. Based on this study the concept of the “endogenous pyrogen” developed. Intensive research focused on the molecular identification and cloning of putative endogenous pyrogens (9, 10). These molecules are members of the still growing family of cytokines. Not all cytokines have the ability to induce fever. Direct pyrogenic properties have been ascribed to

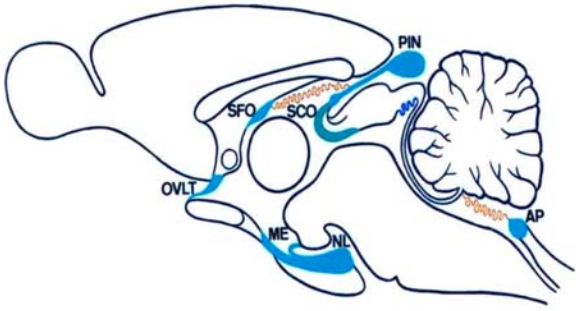

Figure 1. Schematic drawing of a mid-sagittal section through the rat brain; areas which lack a tight blood-brain barrier are indicated by blue color (AP = area postrema; ME = median eminence; NL = neural lobe of the pituitary; OVLT = vascular organ of the lamina terminalis; PIN = pineal organ; SCO = subcomissural organ; SFO = subfornical organ).

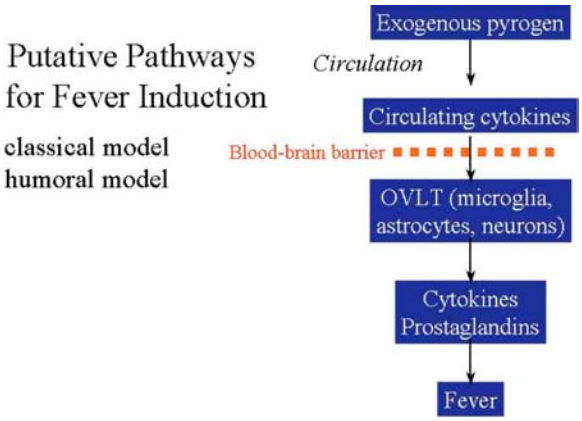

Figure 2. Schematic illustration of the classical humoral pathway for the induction of fever and other brain controlled illness responses, in which circulating pyrogenic cytokines play the key role.

interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF), interferons (IFNs), ciliary neutrotrophic factor (CNTF) and macrophage inflammatory protein (MIP) (for review see: 11, 12, 13, 14).

Fever is thought to be induced by the actions of one or several of the endogenous pyrogens on the highly thermosensitive preoptic area or the thermointegrative structures of the anterior hypothalamus. However, the direct access of the large hydrophilic cytokine-proteins to these structures is prevented by the blood-brain barrier. Thus, it has been proposed for more than 20 years that sensory CVOs are target structures for circulating cytokines and can be regarded as "windows to the brain" for the endogenous pyrogens. Much of what we know about the role of CVOs in the CNS responses to inflammation was borne out of research on fever. The first experimental evidence for such a role derived from classical lesion studies. Later on, electrophysiological and molecular-neuroanatomical investigations provided the background for another look at the putative role of sensory CVOs in signaling the brain during systemic inflammation.

Fever is not the only brain-mediated sign of illness during infectious or inflammatory stimulation. Pyrogenic cytokines also induce changes in neuroendocrine activities (namely a stimulation of the hypothalamic-pituitary-adrenal axis), anorexia, adipsia, modifications of sleep patterns and decreases in locomotor activity, libido, social contacts and exploration, symptoms which are collectively termed sickness behavior (15, 16, 17). The neuroanatomical and functional backgrounds for the manifestation of cytokine-mediated signs of illness are thus of basic and clinical interest. The aim of this review is to analyze the putative role of the sensory CVOs as targets for inflammatory signal molecules and the relevance of these specialized brain structures in the aforementioned cytokine-induced sickness responses.

3. LESIONS OF SENSORY CVOs

In 1983 (18) it was reported that electrolytic ablation of that part of the anteroventral third ventricular region of the brain, which contains the OVLT, prevents the febrile response of guinea pigs to systemic administration of bacterial lipopolysaccharide (LPS). Since LPS is a very potent inducer of "endogenous pyrogens", the authors of this study concluded that the humoral pyrogenic signal is transduced from the blood to the brain via the OVLT. At that time IL-1 was regarded as the exclusive endogenous pyrogen. Blatteis and colleagues (18) not only proposed that the OVLT might be the site through which IL-1 passes into the neuropile, but additionally they showed that injections of a purified IL-1-preparation into the medial preoptic area which surrounds the OVLT still caused fever in OVLT-lesioned animals indicating the animals intact capacity to develop fever independent from humoral signals. Also the normal thermoregulatory responses of guinea pigs to changes in ambient temperature were not impaired by lesions of the OVLT (19).
Based on these studies the hypothesis of a humoral signal transfer of a pyrogenic message from the blood to the brain developed. It was proposed that circulating IL-1 enters the perivascular space of the OVLT, binds to specific not yet identified receptors, possibly located on astroglial processes, and induces a central production of prostaglandin E2 (PGE2) which is traditionally regarded as the final mediator of the febrile response in the brain (20, 21, 22). A schematic illustration of the humoral fever-induction pathway is shown in Figure 2.

After the first report of suppression of fever due to electrolytic lesion of the OVLT in guinea pigs (18) similar experiments were performed in other experimental animal species. Stitt (23) also suggested that the OVLT might be the port of entry for the "endogenous pyrogen" into the brain, however, from rather different results. Using small lesions destroying just the ventrorostral part of the OVLT, the febrile response of rabbits and rats to intravenous injection of a purified preparation of human "endogenous pyrogen" was transiently increased rather than suppressed (23). As a possible reason for this discrepancy it was argued that the size and the completeness of an electrolytic lesion of the OVLT is crucial for the abrogation of fever by this procedure. In line with this argument, in two further studies the completeness of an

OVLT-lesion was related to the ability of sheep (24) and guinea pigs (25) to develop fever in response to systemic administration of LPS. The main outcome of both studies was that the reduction of fever by lesions located within the anteroventral region of the third ventricle only then becomes manifest when the vascular plexus of the lamina terminalis, which is defined as the OVLT, is completely included in the electrolytically ablated area. In a later study lesions of the OVLT, or of the SFO, or of the AP were performed in rats (26). Interestingly, only rats with lesions of the SFO displayed reduced LPS-fevers in this study, indicating a possible involvement of this sensory CVO in the response of rats to circulating pyrogen (26).

As already mentioned in the "Introduction", circulating cytokines not only induce fever but a rather complex array of brain-controlled signs of illness including a constant and reproducible activation of the hypothalamic-pituitary-adrenal (HPA) axis (15, 27). A cytokine-induced stimulation of the HPA-axis results in a rise of circulating levels of adrenocorticotrophic hormone (ACTH) and glucocorticoids, cortisol and / or corticosterone, depending on the investigated animal species (27, 28, 29). Based on the observation that IL-1-induced increases in circulating ACTH and corticosterone were significantly depressed by lesions of the OVLT (30) or the AP (31), a role for sensory CVOs in the cytokine-induced stimulation of the HPA-axis has been suggested. It was further speculated that an activation of cells within OVLT, SFO or AP by circulating cytokines might be transmitted to the hypothalamic paraventricular nucleus by direct or indirect neuronal connections to induce the observed stimulation of the HPA-axis (32). The idea that a pyrogenic message of circulating inflammatory signals is transduced from sensory CVOs to the hypothalamus via neuronal connections is supported by the following observation. In rabbits, the febrile responses to intravenous injections of IL-1β or TNFα were attenuated by bilateral transsection of the neuronal connections between the anteroventral region of the third ventricle and the anterior hypothalamus (33).

Recently a reappraisal of lesion experiments dealing with the putative role of CVOs in immune-to-brain signaling was published (34). In this paper special attention was directed to side effects which are caused by electrolytic lesions of the OVLT. In addition to adipsia, hypernatremia or hyperosmolality, which may be related to the functions of the lamina terminalis in electrolyte and water balance, the most pronounced side effect induced by OVLT lesions was a marked and long-lasting hyperthermia by about 2°C. The authors of this study confirmed a lack of fever in response to IL-1β in OVLT-lesioned rats, but they pointed out that the pronounced hyperthermia in these animals might have prevented fever from developing. As a consequence it was suggested that nonlesion techniques or highly sensitive chemical lesioning techniques should be applied to prove a role for single CVOs as routes of immune-to-brain communication (34).

4. SIGNALING SENSORY CVOs BY ENDOGENOUS PYROGENS

Assuming that circulating cytokines induce brain-mediated illness responses by interactions with sensory CVOs, then the following two prerequisites should be fulfilled. First, it should be possible to detect and demonstrate functionally active receptors for inflammatory humoral signals on cells which are located in these CVOs. Second, appropriate cellular responses should develop within the sensory CVOs after exposure to a given inflammatory cytokine, which can be interpreted as a basis for the manifestations of the centrally controlled signs of sickness occuring during systemic inflammation. In most experimental models of systemic inflammation bacterial LPS is used as the exogenous inducer of the inflammatory response. IL-1β, TNFα and IL-6 are regarded as important endogenous mediators of the LPS-induced biological effects. The possible influence of these three cytokines on sensory CVOs will therefore be analyzed predominantly.

4.1. Cytokine receptors in sensory CVOs

The mRNA for the type-1 receptor of IL-1 is constitutively expressed within various brain areas in mice (35) and rats (36, 37) including OVLT and SFO. However, attempts failed to detect a labeling for IL-1-receptor mRNA in cells of non-vascular origin in both of these sensory CVOs. It was therefore suggested that IL-1 does not directly interact with neuronal elements of OVLT and SFO (36). In the AP, the authors of this study in rats (36) observed a diffuse labeling for IL-1 type-1 receptor mRNA distributed within the whole structure of this sensory CVO. This finding was interpreted as an expression of the IL-1 receptor in non-vascular cells, possibly even in neurons, located within the AP.

TNF mediates its biological effects by two functionally distinct receptors, named p55 and p75 according to their molecular weight. Constitutive expression of mRNA for the p55 receptor was detected in sensory CVOs whereas the transcript of the p75 receptor was hardly detectable in the rat brain (38). Interestingly, intravenous administration of TNFα is leading to a pronounced increase in p55 TNF-receptor expression in the OVLT, SFO, and AP, suggesting that the ligand stimulates the upregulation of its own receptor in sensory CVOs (38).

The first step in IL-6-induced signal transduction is the binding of IL-6 to a specific receptor subunit. Then a second signal transducing subunit named gp130 associates with the ligand receptor complex. Together, these three molecules induce signal transduction in the target cells for IL-6 (see Chapter 6). First attempts failed to detect expression of IL-6-receptor mRNA in sensory CVOs under basal conditions (39). However, a later study (40) provided convincing evidence for the presence of the IL-6-receptor and the gp130 signal transducing unit in sensory CVOs. Both of these molecules are constitutively and even strongly expressed in the OVLT, SFO and AP (40). This finding clearly supports the view that sensory CVOs might represent functionally relevant routes of entry for circulating IL-6 into the brain.

4.2. Cellular responses to inflammatory signal molecules in sensory CVOs

There are several possibilities with regard to cellular responses which might be induced by interactions

of circulating cytokines with their specific receptors on cells located in sensory CVOs. Cytokines could directly alter the electrical activity of neurons within the CVOs. On the other hand, a given cytokine may cause the formation of secondary signal molecules by cells of non-neuronal origin which, in turn, might influence neuronal activity in the nearby neighbourhood in a paracrine manner. Fever, anorexia, or stimulation of the HPA-axis are thought to be controlled by hypothalamic neuronal circuits. Sensory CVOs might thus be the sites where the chemical signals of blood-borne cytokines are transduced into neuronal signals which then have to be transmitted to the autonomic hypothalamic control centers.

There is just one *in vitro* study in which the direct effects of cytokines on the electrical activity of OVLT neurons have been investigated in brain slice preparations. In this study almost 50% of all investigated OVLT neurons changed their firing rates under the influence of TNFα or IFNα (41). The authors of this study speculated that these cytokine-induced electrical activity changes could transsynaptically affect hypothalamic thermosensitive neurons and thereby contribute to the generation of fever. In line with this suggestion, Ota *et al.* (42) recorded single neuron activity in the OVLT area in anesthetized rats under the influence of intravenous or intra-arterial injections of IL-1β. Virtually all of the OVLT-neurons, that could be antidromically activated by electrical stimulation of the medial preoptic area or the hypothalamic paraventricular nucleus, altered their activity in response to systemic administration of IL-1β. In a majority of the OVLT-neurons, which were electrophysiologically identified to send their axons to the hypothalamus, the effect of IL-1β was inhibitory. This effect could frequently be antagonized by the application of sodium salicylate, suggesting that the influence of IL-1β on the electrical activity of OVLT-neurons is mediated, in part, by a local formation of prostaglandins (42). This results suggest that circulating IL-1β represents a humoral signal which is, via localized release of prostaglandins, transduced into a neuronal information at the OVLT, which in turn is transmitted to hypothalamic nuclei involved in thermoregulation. Support for the view that the neuronal effects of cytokines in sensory CVOs are mediated by prostaglandins also derives from the influence of a local administration of PGE2 on the electrical activity of OVLT-neurons *in vitro* in brain tissue slices (43). In more than 85% of the investigated OVLT-neurons PGE2 induced pronounced alterations of the discharge rates, excitation or inhibition. Interestingly, a higher incidence of inhibitory responses to PGE2 was observed among warm-sensitive OVLT-neurons (43). This finding is of particular interest since an inhibition of warm-sensitive neurons is regarded as one of the basic neuronal mechanisms for the generation of fever (44, 45). In addition, a high density of PGE2 binding sites has been documented in the anterior wall of the third ventricle where the OVLT is located (46).

The expression of the immediate-early gene c-fos is frequently used as a marker for functionally activated neurons. Detection of c-fos mRNA by *in situ* hybridization, or of the FOS protein by immunocytochemistry are powerful tools to identify cell groups which, after appropriate stimulation, may be linked to functional neuronal circuits. This technique has therefore been used to characterize central autonomic pathways which are activated during fever (47). This approach has also been applied to demonstrate cellular activation in sensory CVOs in response to systemic administration of cytokines. Intravenous injections of TNFα (38) or IL-6 (48), indeed, induced transcription of the c-fos gene in the OVLT, SFO, and AP. Interestingly, c-fos expression in all sensory CVOs occurred within 60 min from the time of IL-6-administration, while it took 3 hours before c-fos expression was observed in response to a systemic challenge with TNF. The authors of this study (38) concluded that the activation of cells within CVOs by TNFα requires the de novo synthesis of other mediators which, in turn, may be responsible for the delayed c-fos expression in these specialized brain areas. The rapid c-fos induction in all sensory CVOs in response to IL-6 is, on the other hand, indicative of a direct stimulatory effect of this cytokine on neuronal activity in OVLT, SFO, and AP (48). Surprisingly, intravenous injections of IL-1 at doses, which induced a pronounced c-fos expression in the hypothalamic paraventricular nucleus failed to cause the appearance of c-fos mRNA or FOS protein in sensory CVOs (49). Only in response to a 10-times higher dose of IL-1β, FOS-immunoreactivity appeared in cell nuclei of the OVLT. Even with the high dose of IL-1 just single FOS-signals were observed in the AP, while there appeared numerous FOS-positive nuclei in the nearby nucleus of the solitary tract (49). In summary, there seems to be a complex pattern of time- and dose-dependent c-fos expression in sensory CVOs stimulated by circulating cytokines. The temporal and spatial pattern of c-fos expression is determined, in addition, by the route of pyrogen administration, the pyrogen type, and the species tested, since fever onset latencies and febrile courses also vary in relation to these variables. In general, the results from c-fos expression studies can be interpreted as evidence for direct or indirect neuronal activation in sensory CVOs by putative endogenous pyrogens.

Finally, the results of some *in vivo* studies should be mentioned, which deal with the question of whether the actions of circulating pyrogens in single CVOs are involved in the manifestation of fever. Using *in vivo* microdialysis, Fewell *et al.* (50) measured increased levels of PGE2 in the OVLT-region in response to intravenous injection of IL-1β. Under conditions of a natural suppression of fever near term of pregnancy (51) the IL-1β-induced rise of PGE2 within the OVLT is abrogated (50). This observation supports the electrophysiological studies mentioned above (42, 43), in which neuronal responses to IL-1β in the OVLT seemed to be mediated by prostaglandins. In another study (52) LPS-induced fever could be attenuated by local administration of IL-1-receptor antagonist into the SFO, indicating a fever-promoting biological action of IL-1 in this sensory CVO. On the other hand, it should be noted that attempts failed to demonstrate binding of radiolabelled IL-1 within the OVLT after its injection into the carotid artery (53). Continuous infusion of PGE2 into the carotid artery produced a fall in body temperature rather than a

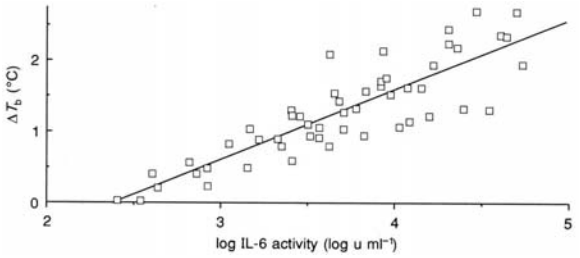

Figure 3. Correlation between bioactive IL-6 in the blood and febrile temperature changes in guinea pigs. Logarithmic values of individual levels of bioactive IL-6 in plasma 60 or 180 min after injection of bacterial LPS versus the corresponding individual increases of body core temperature from the baseline temperature [from (61), with permission from The Physiological Society].

fever in guinea pigs (54). It has therefore been questioned by the authors of this study if cellular elements of the OVLT respond to circulating PGE2 in a pro-pyretic manner under *in vivo* conditions.

Kluger (11) postulated a number of criteria which should be fulfilled by a given endogenous substance to be accepted as a putative signal to the brain during systemic inflammation. With regard to the febrile response, which constantly develops during infection or inflammation, one of these criteria is that the release of the putative endogenous pyrogen should have some quantitative relationship to the rise in body temperature. As already mentioned above, in most experimental studies on systemic inflammation LPS from gram-negative bacteria is used as the exogenous inducer of the acute-phase response. TNFα, IL-1β, and IL-6 are produced and released with very characteristic strength and kinetics during LPS-induced fever. TNF only transiently appears in the bloodstream for a short period with a rapid and brief peak occurring about 60 min after LPS-injection (11, 28, 29, 55). Dependent on the administered dose of LPS, just small traces of IL-1β are measurable in blood plasma. Frequently, IL-1 even escapes its detection as a humoral signal during a systemic LPS-induced inflammatory response (11, 28, 56). IL-6 is the only inflammatory cytokine that can be measured in significant quantities in the blood during the time course of fever (11, 55). Thus, IL-6 fulfils an important criterion to act as a humoral signal to the brain during systemic inflammation. If IL-6 would act as a circulating messenger between the activated immune system and the brain at the level of the sensory CVOs, it might be possible to demonstrate a direct influence of this cytokine on cellular elements located within these brain structures. This issue and the general role of IL-6 in systemic inflammation will therefore be addressed.

5. THE ROLE OF INTERLEUKIN-6 IN SYSTEMIC INFLAMMATION

IL-6 is produced by numerous cell types during infection, inflammation, trauma, and other immunstimulatory events. Elevated circulating levels of IL-6 have therefore been associated with the state of a given disease (57). On the one hand, IL-6 promotes inflammatory responses due to its stimulatory effects on T-cell-activation, B-cell-differentiation, and the induction of acute-phase proteins. On the other hand, IL-6 also counteracts the manifestation of inflammatory responses and has protective functions during severe states of disease (58).

Evidence for a role of circulating IL-6 in fever derives from observations that there is a very good correlation between plasma concentrations of IL-6 and the magnitude of fever in human patients (59) and in experimental animals (55, 60, 61). An example for the rather excellent correlation between plasma IL-6 and the febrile change of body temperature is shown in Figure 3.

Figure 3 summarizes data from a number of experiments in which fever and plasma concentrations of IL-6 have been measured in response to systemic injections of LPS, repeated in intervals of 3 days in guinea pigs (61). All individual febrile temperature changes have been related to the corresponding logarithmic values of IL-6 in plasma. The close correlation between both of these variables (correlation coefficient = 0.862; p = 0.0012) suggests that circulating IL-6 is an appropriate candidate to act as a humoral signal that may be sensed by OVLT, SFO, or AP. The correlation between febrile body temperature changes and plasma IL-6 alone does not prove that fever is really caused by circulating IL-6. Indeed, it has been reported that systemic administration of IL-6 alone has rather moderate pyrogenic effects in several species of experimental animals (62, 63, 64, 65). However, evidence for an important role for IL-6 in fever derives from the following findings. The febrile response to LPS or IL-1β is abrogated in IL-6-deficient ("knockout") mice (66). These mice, on the other hand, develop fever to central administration of exogenous IL-6, suggesting that IL-6 gene expression is essential for the manifestation of a febrile response. Cartmell *et al.* (64) injected LPS into a subcutaneous air pouch in rats and documented that IL-6 is the only inflammatory cytokine that enters the systemic circulation from the site of local inflammation. Systemic treatment with antibodies directed against rat IL-6 suppressed the febrile response of rats to intrapouch injections of LPS. The authors of this study postulated that circulating IL-6 plays an important role in the manifestation of fever, but needs other factors to elicit a full febrile response (64). In other words, IL-6 seems to activate fever-inducing brain mechanisms by acting in concert with other pyrogenic molecules.

6. TRANSCRIPTIONAL ACTIVATION OF CELLS WITHIN SENSORY CVOs BY CIRCULATING IL-6

As already mentioned above (see Chapter 4.1.), IL-6 not only needs its specific cognate receptor subunit but also the gp 130 signal-transducing element. The group of cytokines which signal through the gp 130 receptor subunit includes IL-6, IL-11, ciliary neurotrophic factor (CNTF), leukemia inhibiting factor (LIF) and leptin (67, 68, 69). This group of cytokines has therefore been called the "IL-6 family of cytokines". The signal transduction induced by the interactions of IL-6, the IL-6-receptor, and gp 130 can briefly be summarized as follows.

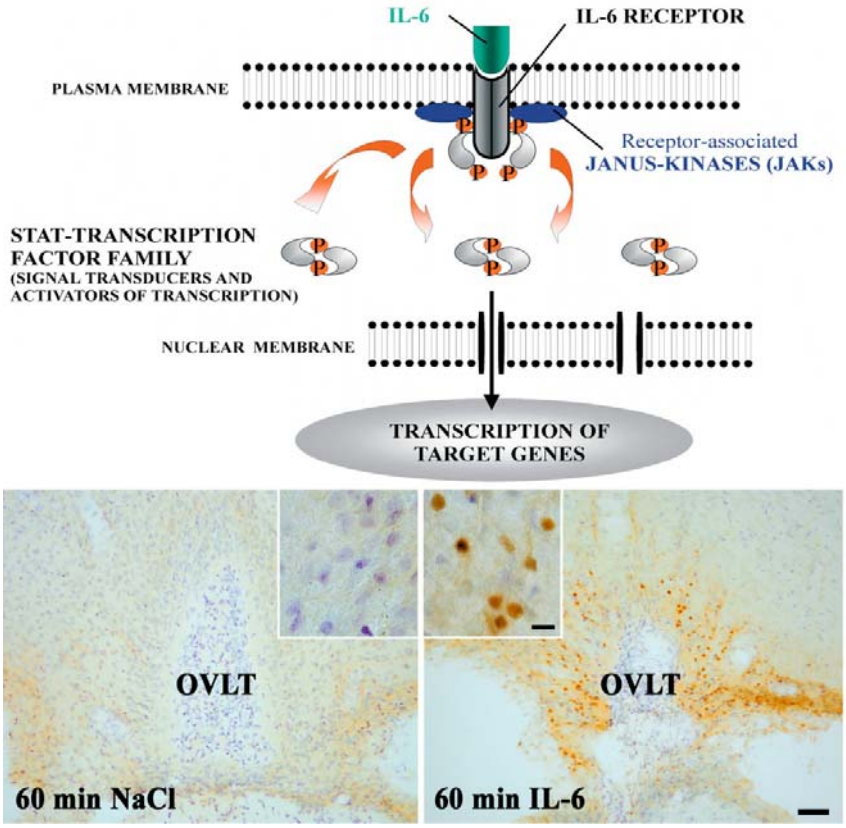

Figure 4. Upper part: schematic illustration of the IL-6-induced signal transduction in target cells (see text for further details). Lower part: Microphotographs of brain sections from the OVLT of rats, 60 min after intraperitoneal injection of sterile saline (left) or IL-6 (right). The specific STAT3 immunoreactivity is depicted from the brown reaction product, due to the visualization via diaminobenzidin conversion. STAT3 labeling after IL-6 application is shown at higher magnification in the inset compared with blue colored cell nuclei (cresyl violet counterstaining) of the respective control situation [for further details see (65), with permission from The American Physiological Society].

Homodimerization of gp 130 to the ligand-receptor complex activates a specific signal transduction pathway, the so called Janus kinase-signal transducer and activator of transcription (JAK-STAT) signaling cascade. First step within this cascade is the activation of gp 130 associated cytoplasmatic tyrosine kinases (JAKs). Then, the STAT3 isoform of this group of transcription factors is phosphorylated, dimerizes and translocates into the nucleus of the IL-6-stimulated cell, where it regulates gene expression by binding to specific gene promoters (70). The IL-6-induced signal transduction in a target cell is schematically illustrated in the upper part of Figure 4.

Phosphorylated STAT3 thus accumulates in nuclei of IL-6-stimulated cells and can be visualized by means of immunohistochemistry. Hübschle *et al.* (71, 72) reported that intracerebroventricular microinjections of IL-6 or leptin caused distinct and very characteristic, cytokine-specific patterns of nuclear STAT3 translocation in brain areas involved in fever (IL-6) or body weight regulation (leptin). Thus, STAT3 immunohistochemistry proved to be an excellent tool to demonstrate genomic activation of brain cells induced by IL-6. If IL-6 is a critical circulating mediator for an inflammatory stimulation of sensory CVOs, it should be possible to demonstrate genomic activation of cellular elements within CVOs in relation to a rise of IL-6 in the blood.

The first indication that circulating IL-6 might activate the illustrated signal transduction pathway at the level of sensory CVOs derives from a study by Konsman *et al.* (73). The authors of this study described for the first time a nuclear STAT3 translocation in cells of the OVLT after treatment of rats with bacterial LPS. Recently, a study from our laboratory was published in which the time course of nuclear STAT3 translocation in all sensory CVOs was investigated in response to intraperitoneal injections of LPS or IL-6 in rats (65). In addition, circulating levels of IL-6 were measured at all investigated time intervals from the administration of both pyrogens, and the time dependency of specific nuclear STAT3 translocation was quantitatively verified by counting the STAT3 labeled cell nuclei in the sensory CVOs. An example from this study is shown in the lower part of Figure 4. Specific STAT3 immunoreactivity (brown color) appears in cell nuclei within the OVLT of an IL-6-treated rat, compared to the absence of nuclear STAT3 signals in a saline injected control animal. The quantitative evaluation of this study can be summarized as

follows [for more details see: (65)]. Intraperitoneal administration of LPS (100 µg/kg) or IL-6 (45 µg/kg) caused an almost exclusive nuclear STAT3 translocation in cells of the OVLT and SFO. There was a strict temporal pattern of the observed nuclear translocation of STAT3 in both sensory CVOs. In response to LPS, nuclear STAT3 signals appeared not earlier than two hours after injection of the exogenous pyrogen and disappeared again to a high degree within the following 60 minutes. However, nuclear STAT3 translocation in the same brain structures already occurred one hour after IL-6 application, which also disappeared within the following hour. This time course of nuclear STAT3 translocation excellently corresponded to the circulating levels of IL-6, which reached high and similar values one hour after IL-6- and two hours after LPS treatment. Thus, the results of this study provided clear evidence for a genomic activation of cellular elements within sensory CVOs by circulating IL-6. The constant presence of IL-6 in the blood during systemic inflammation or trauma can therefore be accepted as a clear indication for a role of this cytokine to represent a circulating signal which is in the rat, indeed, sensed by specialized brain structures, the OVLT and the SFO.

Which brain structures can be reached and genomically influenced by systemic IL-6 seems to depend on the strength of the inflammatory stimulus and thereby on the concentration of circulating IL-6, and on the investigated species of experimental animals. Using a higher dose of LPS in rats (250 µg/kg; intraperitoneal route of administration), Gautron *et al*. (74, 75) observed nuclear STAT3 signals not only in OVLT and SFO, but also in the median eminence, in the pituitary, and even in some hypothalamic nuclei. In guinea pigs, intraperitoneal injection of just 30 µg/kg LPS induces strong nuclear STAT3 translocation in OVLT and SFO (like in rats) and very pronounced also in the AP, in the ventromedial preoptic nucleus, and in the hypothalamic supraoptic nucleus (76). It should, however, be noted that the LPS-induced circulating levels of bioactive IL-6 were almost 10-times higher in guinea pigs when compared to rats, even though the injected LPS-dose was substantially lower [30 µg/kg in guinea pigs versus 100 µg/kg in rats (65, 76)]. Again, this observation indicates that there is a correlation between the amount of circulating IL-6 and the strength of nuclear STAT3 signals in sensory CVOs and some other brain structures. Another important question which derives from these studies concerns the phenotype of cells within sensory CVOs which are activated by blood borne IL-6. With regard to LPS-induced nuclear STAT3 translocation, Gautron *et al*. (74) reported an almost exclusive location of nuclear STAT3 signals in astrocytes. We also identified a population of STAT3 positive cells as astrocytes, which showed up in OVLT and SFO in response to LPS as well as IL-6 (77). However, there was also a significant population of cells responding with nuclear STAT3 translocation to LPS or IL-6, which were not labeled by a specific cell marker for astrocytes and the phenotype of which is unclear at the moment (77). The functional relevance of IL-6-induced nuclear STAT3 translocation in astrocytes and other cell types located within sensory CVOs will have to be elucidated. Genes which are expressed or upregulated in the brain by systemic inflammation include COX-2 (78) or the isoforms of NO-synthases (79). It is not clear at present, if the transcription of these genes is directly or indirectly related to IL-6-induced nuclear translocation of STAT3 in cells within sensory CVOs. It will, however, be a task for future studies to characterize the nature and temporal patterns of activated genes in these cells during systemic inflammation.

7. SIGNALING SENSORY CVOs BY EXOGENOUS PYROGENS

The formation of cytokines and their appearance in the blood after a challenge with bacterial LPS requires some time. It has been stated that sometimes the LPS-induced onset of fever (80) or the LPS-induced activation of the HPA-axis (28) can precede the systemic production of considerable amounts of inflammatory cytokines. On the other hand, so called "anti-cytokine strategies" with the aim to neutralize a given cytokine or to antagonize the biological action of a cytokine often result in attenuation or suppression of later phases of fever, while the early phase of the febrile response remains manifest (14, 81). If the early phase of LPS-fever develops independently from circulating cytokines, what kind of signal then mediates the quick onset and the development of the early stage of fever? One alternative and rapid signal pathway between the activated immune system and those parts of the brain which are involved in fever induction would be via stimulation of afferent nerves (14, 82, 83, 84, 85). Additionally, experimental evidence for a direct stimulation of cells within sensory CVOs by exogenous pyrogens, namely by LPS, has accumulated. Using *in situ* hybridization and immunohistochemical techniques, Nakamori *et al.* (86, 87) demonstrated a localized formation of IL-1β in the OVLT in rabbits, in response to intravenous injection of a moderate dose of LPS. With similar approaches, Breder *et al.* detected induction of TNFα in the OVLT and AP during the early phase of LPS-induced fever in mice (88). While peripheral injections of septic doses of LPS induced global expression of proinflammatory cytokines in the brain, subseptic, but fever-inducing LPS-doses induced cytokine expression only in the choroid plexus, in the meninges and prominently in sensory CVOs (40, 89). The authors of these studies postulated a direct influence of LPS on cellular elements within the CVOs with the consequence of a local formation of cytokines at the interface between blood and brain. The explanation for the localized formation of cytokines at the level of the sensory CVOs was provided in a number of convincing studies (90, 91, 92). LPS exerts its action on mononuclear phagocytes, predominantly the induction of a "cytokine cascade", via CD14 and the so called Toll-like receptors. CD14 as well as Toll-like receptor type 4 are constitutively expressed in sensory CVOs; circulating LPS is even able to upregulate the expression of CD14 and to induce the expression of Toll-like receptor type 2 within these specialized brain structures (92). Therefore, without having direct access to the brain parenchyma, an exogenous pyrogen such as LPS, is sensed by the CVOs and triggers pronounced brain-intrinsic responses possibly even prior to the appearance of endogenous pyrogens (cytokines) in the blood.

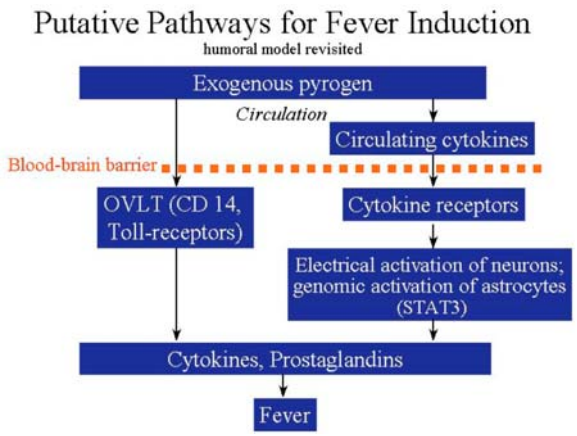

Figure 5. Expanded schematic illustration of the classical humoral pathway for the induction of fever.

8. FINAL CONCLUSIONS

Around the time, when Hellon and Townsend (93) originally postulated that circulating „endogenous pyrogen" might gain access to the brain via the OVLT, the first experimental study on this question was published (18). From this and numerous other studies the model of a humoral pathway for fever induction derived (see Figure 2). This model is still useful and valid and, according to the increased knowledge described in this review, more light has been thrown on the cellular and molecular interactions between circulating pyrogens and their targets within sensory CVOs. Thus, an expansion of the humoral fever-induction pathway is illustrated in Figure 5.

In Figure 5 it is indicated that receptors for exogenous (LPS) or endogenous progens (cytokines) have been identified within the OVLT and other sensory CVOs and that responses of cells within the CVOs to the respective pyrogens have been characterized. With regard to the manifestation of fever, much less is known about the steps which follow the activation of cellular elements within sensory CVOs by exogenous or endogenous pyrogens. In this context, a lot of importance has been attached to the ventromedial preoptic area (VMPO) which is located in close vicinity to the OVLT (47). The authors of this paper hypothesize that OVLT and VMPO form a functional unit in which cells within the VMPO represent the key site for the initiation of fever during systemic inflammation. The coupling of this brain site to the activation of fever producing autonomic pathways seems to be mediated by PGE2 (94) via its EP4 receptor subtype (95). In contrast, Nakamura *et al.* (96) suggested an alternative efferent fever pathway that arises from the medial preoptic area, again in close vicinity to the OVLT, but distinct from the VMPO area. This pathway seems to use the PG-receptor subtype EP3 to elevate body temperature via activation of the brown adipose tissue, a major thermoeffector organ in rats. Indeed, using the viral tracing technique combined with immunocytochemical detection of the EP3 receptor we recently demonstrated an efferent neuronal pathway from the medial preoptic area, in particular its median preoptic nucleus, to the interscapular brown adipose tissue (97). A colocalization of virus protein with the EP3 receptor was also found in some neurons of the OVLT itself (97). This neuronal chain might thus be regarded as the efferent part of the thermoregulatory reflexes which are activated by interactions of circulating pyrogens with cells located within the OVLT / medial preoptic area.

Finally, it should be mentioned that there is not just one defined pathway for the induction of fever and other brain-controlled signs of illness (13, 14, 80, 82, 83, 84, 85). All the findings including our own data, which form the basis for the content of this paper derive, from selected experimental conditions, i.e. a given pyrogen, a given dose of this pyrogen, a given route of pyrogen administration, and a given animal species. The major goal of this article was to focus on the possible participation of sensory CVOs as receptive sites for inflammatory signals. Alternative pathways of immune-to-brain communication are analyzed in other chapters of this special issue.

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