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Review

Cytokine-induced sickness behaviour: a neuroimmune response to activation of innate immunity

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Abstract

Sickness refers to a coordinated set of subjective, behavioural and physiological changes that develop in sick individuals during the course of an infection. These changes are due to the effects of interleukin-1 (IL-1) and other proinflammatory cytokines on brain cellular targets. Sickness behaviour is mediated by proinflammatory cytokines that are temporarily expressed in the brain during infection. These centrally produced cytokines are the same as those expressed by innate immune cells and they act on brain receptors that are identical to those characterized on immune cells. Primary afferent nerves represent the main communication pathway between peripheral and central cytokines. Proinflammatory cytokines modulate learning and memory processes. The expression and action of proinflammatory cytokines in the brain in response to peripheral cytokines are regulated by various molecular intermediates including anti-inflammatory cytokines such as interleukin-10 (IL-10) and the IL-1 receptor antagonist (IL-1ra), growth factors such as insulin-like growth factor-1 (IGF-1), hormones such as glucocorticoids and neuropeptides such as vasopressin and alpha-melanotropin.

Keywords: Sickness behaviour; Cytokines; Inflammation; Brain; Afferent nerve; Pain; Sleep; Learning and memory

Contents

1. Introduction . . . 400
2. Cytokine-induced sickness behaviour is an adaptive response to infection . . . 400
3. Peripherally produced cytokines act in the brain on brain cytokine receptors to induced sickness behaviour . . . 402
4. The peripheral immune message is transmitted to the brain via both neural and humoral communication pathways . . . 404
5. Cytokines play a role in learning and memory . . . 406
6. The behavioural effects of cytokines are tightly regulated . . . 407
7. Conclusions and perspectives . . . 408
References . . . 409

1. Introduction

Infection by pathogenic microorganisms triggers in the host a set of immune, physiological, metabolic, and behavioural responses known to pathologists as the acute phase reaction. These responses are mediated by the activation of innate immune cells that recognize pathogen-associated molecular patterns via membrane Toll-like receptors. A typical pathogen-associated molecular pattern is represented by lipopolysaccharide (LPS), the active fragment of Gram negative bacteria. LPS binds to Toll-like receptor-4 on monocytes and macrophages, which

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activates complex intracellular signalling pathways including docking proteins and phosphorylation cascades, resulting in the activation of nuclear transcription factors. The proinflammatory cytokines that are produced as a result of the activation of a nuclear factor kappaB signalling pathway are part of a cytokine network that includes cytokines opposing the production and action of proinflammatory cytokines. These anti-inflammatory cytokines can be specific of a given proinflammatory cytokine such as the interleukin-1 receptor antagonist (IL-1ra) that binds specifically to interleukin-1 (IL-1) receptors, or act on the production and action of a number of proinflammatory cytokines, such as interleukin-10 (IL-10) that down regulates via its own receptors the production of IL-1, interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α).

The systemic administration of LPS or recombinant proinflammatory cytokines such as IL-1 to healthy laboratory animals or human volunteers triggers the whole set of responses that are characteristic of the acute phase reaction, including its central component in the form of fever, activation of the hypothalamic-pituitary-adrenal axis and the behavioural symptoms of sickness. The same clinical signs can be induced by injection of LPS or interleukin-1 into the lateral ventricle of the brain, which indicates that the brain is able to recognize immune molecular signals.

The aim of this review paper is to summarize the main findings that have been obtained during the recent years on the mechanisms and biological significance of cytokine-induced sickness behaviour.

2. Cytokine-induced sickness behaviour is an adaptive response to infection

Typical symptoms of sickness include weakness, malaise, listlessness and inability to concentrate. Sick individuals are somewhat depressed and lethargic. They show little interest in their surroundings and stop eating and drinking. This constellation of non-specific symptoms is collectively referred to as "sickness behaviour" (Hart, 1988). Since symptoms of sickness behaviour do not help to recognize which pathological process patients are suffering from, most physicians do not pay much attention to them in contrast to disease-specific signs that permit diagnosis. At the best, sickness behaviour is considered as an uncomfortable, but rather banal, component of the pathogen-induced debilitative process. In contrast to this commonly held view, Hart (Hart, 1988) proposed that the behavioural symptoms of sickness represent, together with the fever response, a highly organized strategy of the organism to fight infection. When put forward, this hypothesis was coherent with the already recognized role of fever in the host response to pathogens (Kluger, 1979). In physiological terms, fever corresponds to a new homeostatic state that is characterized by a raised set-point of body temperature regulation. A feverish individual feels cold at usual thermoneutral environments. Therefore it not only seeks warmer temperatures but also tries to enhance heat production (increased thermogenesis) and reduce heat loss (decreased thermolysis). The higher body temperature that is achieved during fever stimulates proliferation of immune cells and is unfavourable for the growth of many bacterial and viral pathogens. In addition, the reduction of zinc and iron plasma levels that occurs during fever decreases the availability of these vital elements for growth and multiplication of microorganisms. The adaptive nature of the fever response is apparent from studies showing that organisms infected with a bacteria or virus and unable to mount an appropriate fever response because they are kept in a cold environment or are treated with an antipyretic drug, have a lower survival rate than organisms which develop a normal fever (Kluger, 1986).

The amount of energy that is required to increase body temperature during the febrile process is quite high since, in human beings, metabolic rate needs to be increased by 13% for allowing a raise of 1 °C in body temperature. Because of this high metabolic cost of fever, there is little room for activities other than those favouring heat production (e.g., shivering) and minimizing thermal losses (e.g., rest, curl up posture, piloerection). Since sickness behaviour is very often accompanied by pain, it has been proposed that this response is an integral part of sickness behaviour if not the main determinant of it (Watkins and Maier, 2000). According to these authors, cytokine-induced hyperalgesia would be advantageous to the survival of the organism, by directing recuperative behaviours (e.g., licking and protection of the affected bodily site) to the site of injury or infection. Furthermore, by encouraging the organism to curl up and remain immobile, exaggerated pain would also serve to save energy.

Within this context, sickness behaviour can be seen as the behavioural expression of a highly organized strategy that is critical to the survival of the organism in face of microbial pathogens, just like fear in face of a predator. If this hypothesis is correct, sickness should have motivational properties, i.e., sick individuals should be able to reorganize their behaviour depending on its consequences and the internal and external constraints they are exposed to. This flexibility is characteristic of motivated behaviour. Motivation is defined here as a central state that reorganizes perception and action. In order to escape a potential threat, a fearful individual must be attentive to everything that can occur in his environment so as to be able to exhibit at the right time the most appropriate defensive behavioural pattern that is available in his behavioural repertoire. In other words, a motivational state does not manifest itself by fixed behavioural patterns. On the contrary, motivation uncouples action from stimulus conditions and enables to select the appropriate strategy depending on the eliciting situation.

The first evidence that sickness behaviour is the expression of a motivational state rather than a consequence of weakness and physical debilitation was provided by Miller (Miller, 1964). While he was working on the mechanisms of thirst, he was struck by the observation that thirsty rats injected with endotoxin stopped bar pressing for water but, when given water, drank it although to a lesser extent than normally. This effect was not specific to the thirst motivation since the endotoxin treatment also reduced the amount of food eaten and even blocked responding in rats trained to press a bar for the rewarding effects of electrical stimulation in the lateral hypothalamus (self-stimulation). Interestingly enough, when rats were trained to turn off an aversive electrical stimulation in this neural structure, endotoxin also reduced the rate of responding, but to a lesser extent than bar pressing for a rewarding brain stimulation. To demonstrate that this range of responses to endotoxin was the result of the elicitation of a sickness motivational state, Miller administered endotoxin to rats that were trained to obtain restful periods by pressing a lever when placed in a rotating drum. Endotoxin-treated rats increased their response rate in response to the endotoxin treatment. The mere fact that endotoxin treatment decreased or increased behavioural output depending on its consequences gives strong support to the motivational interpretation of the behavioural effects of such a treatment. The problem, however, with Miller's observation is that the corresponding findings were reported in a review paper but never published as original results.

The motivational interpretation of sickness behaviour was not submitted to test for 30 years after this insightful observation, before Aubert et al. (1995a,b, 1997) initiated an extensive series of experiments to test it. An important characteristic of a motivational state is that it competes with other motivational states for behavioural output. It is normally not possible to search for food and court a potential sexual partner at the same time since the behavioural patterns of foraging and courtship are not compatible with each other. The normal expression of behaviour therefore requires a hierarchical structure of motivational states which is updated contingent on urgencies that occur in the internal and external milieu. When an infection occurs, the sick individual is at a life or death juncture and its physiology and behaviour must be altered so as to overcome the disease. However, this is a relatively long-term process which needs to make room to more urgent needs when necessary. To take an analogy, it is obvious that if a sick person lying in his bed hears a fire alarm ringing in his house and sees flames and smoke coming out of the basement, he will momentarily overcome his sickness behaviour to try to escape danger. In motivational terms, fear competes with sickness and, in behavioural terms, fear motivated behaviour takes over sickness behaviour. An example of this competition between sickness and other motivational states is given by the observation that the depressing effects of LPS on behaviour of lactating mice were abrogated when LPS-treated mothers had to retrieve their pups after they had been removed from the nest and scattered throughout the home cage (Aubert et al., 1997). Additionally, LPS-treated mothers also engaged in nest building when the motivation to build a nest was increased by placing the mother with her litter at an ambient temperature of 6 °C, whereas nest building did not occur when the animals were tested at 22 °C.

From an adaptive point of view, the anorexic effect of cytokines is difficult to reconcile with the pyrogenic activity of these molecules. The decrease in food intake that accompanies fever appears to be inconsistent with the enhanced energy requirement of thermogenesis. To resolve this paradox, it has been proposed that cytokine-induced anorexia spares energy required for foraging and prevents a weakened organism to run into the risk of being exposed to a predator during the search of food. From this perspective, it can be predicted that cytokines should be more effective to suppress the foraging than the consummatory components of food intake. This appears to be the case since, as previously mentioned, LPS- or IL-1-treated animals stopped pressing a lever for food but still ate the food pellets which were delivered independently of their behaviour (Aubert et al., 1995b).

Food intake can be affected by cytokines not only in a quantitative but also in a qualitative way. It has been amply demonstrated that when rats are given the opportunity to select components of their diet, their selection pattern reflects the organism's nutritional and energetic requirements. To determine whether this selection pattern was altered during sickness, rats were submitted to a dietary self selection protocol in which they had free access to carbohydrate, protein and fat diets for 4 hours a day (Aubert et al., 1995a). After a 10-day habituation to this regimen, they were injected with LPS or IL-1β. Under the effect of this treatment, they decreased their total food intake but reorganized their self-selection pattern so as to ingest relatively more carbohydrate and less protein whereas fat intake remained unchanged. This change in macronutrient intake contrasted with the increased fat intake that occurs in rats exposed to cold. Although eating fat would be a better way for feverish animals to fulfil their increased energy requirements, it would not be of much use since cytokines have profound metabolic effects resulting, among others, in increased lipolysis and hypertriglyceridemia. Under these conditions, an increased intake of fat would actually be counterproductive since it would further enhance hyperlipidemia without positively contributing to lipid metabolism.

3. Peripherally produced cytokines act in the brain on brain cytokine receptors to induced sickness behaviour

The first evidence in favour of a possible role of cytokines in sickness behaviour came from clinical trials

with purified or recombinant cytokines in the treatment of intractable viral diseases and cancer. Patients injected with these molecules develop flu-like symptoms. Upon repetition of injections, some of them can display acute psychotic episodes characterized by depression or excitation (Capuron and Dantzer, 2003). Fortunately, these symptoms spontaneously regress on cessation of treatment. Toxicological studies carried out on laboratory animals confirmed the neurotropic activity of recombinant cytokines such as IL-1β and TNF-α. Animals injected acutely or chronically with these molecules usually appear lethargic, anorexic and withdrawn from their environment (Kent et al., 1992b). The same effect is observed when animals are injected with LPS.

More objective studies of the sickness inducing properties of cytokines have been based on changes in locomotor activity, social activities and food intake that develop in cytokine-treated animals (Dantzer, 2001a,b). Adult animals presented with juveniles of the same species spontaneously investigate these social stimuli using mainly olfactory cues. This form of social behaviour keeps being expressed at a high level as long as unfamiliar juveniles are presented. Systemic administration of LPS, IL-1β and TNF-α to adult rats or mice decreases the duration of social investigation. These effects are slow to appear since they do not develop before 2–4 h after injection. They last for 4–6 h and usually dissipate by 24 h. Systemic administrations of IL-1 and TNF-α suppress feeding and drinking. This effect has been observed using various measurements of food and water intake in ad libitum as well as deprived conditions (Kent et al., 1996; Plata-Salaman, 1996). A reduction in food intake can occur as a result of different mechanisms, so that all anorexic cytokines do not necessarily share the same mode of action. For example, low doses of IL-1β reduced both meal size and meal duration whereas higher doses also decreased meal frequency. In contrast, interferon reduced both meal size and meal duration but not meal frequency (Plata-Salaman, 1996). A direct comparison of the effects of administration of IL-1β on social exploration and food motivated behaviour in rats revealed that the time course of action is different according to the behavioural end-point since decreases in food intake typically develop within one hour after injection (Kent et al., 1996). This difference is due to the fact that the effects of IL-1 on food intake are mediated both peripherally and centrally whereas those of IL-1 on social exploration are mediated only centrally (see Section 3).

An important component of sickness behaviour is the increased somnolence that occurs during infectious episodes. The evidence in favour of somnogenic properties of cytokines came originally from the observation that the endogenous molecules which are responsible for the increased sleepiness induced by sleep deprivation in experimental animals are bacterial cell wall products that are known as muramyl peptides (Krueger and Majde, 1994). Intravenous injection of IL-1β or TNF-α to rabbits induces dose-related increases in the amount of time spent in slow-wave sleep (Krueger et al., 2003). In rats, the effects of IL-1β on the architecture of sleep is more complex and depends on the circadian phase and the dose (Grazia de Simoni et al., 1995). For instance, low doses of IL-1β increased slow-wave sleep during the light period whereas higher doses increased wakefulness. In view of the somnogenic properties of cytokines, it is important to note that the behaviourally depressing effects of cytokines do not necessarily result from the intrusion of sleeping episodes in the time budget of sick animals. For instance and as mentioned earlier, LPS-treated rats stopped lever pressing in an operant conditioning procedure in which the presentation of food was contingent on the intrusion of an operant lever in the cage, but still ate the delivered food (Aubert et al., 1995b). In the same manner, in the social exploration test, IL-1β-treated rats still responded to the juvenile when it came in contact with them but they did not follow it.

One of the cardinal symptoms of inflammation is pain. Because of the key role played by cytokines in inflammation, their effects on pain sensitivity have been assessed using different experimental paradigms. Hyperalgesia in response to IL-1β has been observed in four different model systems: the rabbit isolated ear perfusion model (Schweizer et al., 1988), the paw pressure test in rats (Ferreira et al., 1988), the tail-flick test and the formalin test (Watkins et al., 1994b) (Wiertelak et al., 1994). In the rabbit isolated ear perfusion model, IL-1 enhanced the blood pressure effects induced by acetylcholine, which is typical of pain-inducing compounds. Intraplantar injection of IL-1 also resulted in an increased sensitivity to paw pressure (Fukuoka et al., 1994). This effect was not restricted to the site of injection since it was also observed in the contralateral paw. A similar hyperalgesia was also observed in the tail-flick test and the formalin test. Rats injected intraperitoneally with LPS or IL-1β displayed prolonged hyperalgesia which developed within 5–10 min following injection and lasted for at least 1 h. Although all these results are strongly suggestive of an enhancing influence of cytokines on pain sensitivity, analgesia following cytokine injection has also been observed using the hot plate test and the phenylquinone writhing test. A possible explanation for these contradictory effects is the difference in time of testing after injection rather than the technique used to assess pain sensitivity. In a systematic study of the time course of the modulating influence of LPS on pain sensitivity in the hot plate test, LPS was found to first increase pain sensitivity. This effect was followed by an analgesic response beginning at 2 h and disappearing at 30 h after administration of LPS (Yirmiya et al., 1994). The delayed analgesic effects were certainly mediated by the release of endogenous opioids since they were blocked by administration of naltrexone. Proinflammatory cytokines can also elicit allodynia, i.e., a pain response to a normally innocuous stimulus. A typical case of allodynia is the increased sensitivity to rectal distension that occurs in LPS-treated

rats (Coelho et al., 2000). Rectal distension with a volume of 0.8 ml but not 0.4 ml elicited pain in rats, based on the occurrence of abdominal contractions. However, rats that had been injected with i.p. LPS 12 h before became sensitive to rectal distension with a volume of 0.4 ml.

Administration of LPS or inflammatory cytokines into the lateral ventricle of the brain induces the same general effects as systemic injection of these products, including fever, social withdrawal, anorexia, and sleep (Dantzer, 2001a,b; Kent et al., 1992b). Hyperalgesia and allodynia can also be observed when cytokines are administered centrally. In accordance with the effects observed at the periphery, intracerebroventricular administration of IL-1β produced thermal hyperalgesia in rats (Watkins et al., 1994b). These effects appear to be of central origin since administration of the same dose of IL-1β at the periphery was totally ineffective. However, hyperalgesia in response to central injections of cytokines is not always constant. Analgesia can also be obtained when IL-1 and TNF-α are administered into the lateral ventricle of the brain or into specific brain nuclei. Whether hyperalgesia or analgesia is observed depends on the dose and the exact site of administration of the cytokine under study. For example, Sacerdote et al. (1992) reported that doses of IL-1β greater than 5–10 ng were ineffective to induce analgesia. Oka et al. (1995) observed that IL-1β was hyperalgesic when it was injected into the preoptic nuclei of the hypothalamus whereas it was analgesic when injected into the ventromedial nucleus of the hypothalamus.

The observation that cytokines are active when injected directly into the brain indicates that cytokine receptors are present in the brain. The expression of cytokine receptors in the brain has actually been demonstrated using binding techniques, molecular biology techniques and immunohistochemistry (Parnet et al., 2002). Brain cytokine receptors are equivalent structurally and functionally to those characterized on peripheral immune and non-immune cells. The cytokine receptors that have been the most intensively studied are those of IL-1, because of the potent effects of this cytokine on the brain. IL-1 receptors are characterized by three extracellular immunoglobulin-like domains and a cytoplasmic Toll domain that is critical in the downstream signalling cascades activated during inflammation (Dunne and O'Neill, 2003; Sims, 2002). The type IL-1 receptor mediates all of the known biological effects of IL-1. The type II IL-1 receptor is a negative regulator of the effects of IL-1 and functions as a decoy receptor. The additional IL-1 receptor accessory protein is necessary for signal transduction but does not bind IL-1. IL-1 receptors are related to the Toll family of receptors by five regions of homology in the cytoplasmic domain that are known as the Toll-IL-1 receptor domain. Binding of IL-1 to IL-1 receptors drives dimerization of the type I IL-1receptor with its accessory protein, followed by recruitment and phosphorylation of receptor-associated kinases via a docking molecule called myeloid differentiation factor 88. Interleukin-1 receptor associated kinase-1 subsequently interacts with the TNF receptor associated factor 6. This is followed by phosphorylation of nuclear factor kappaB inducing kinase, which phosphorylates the inhibitory kappaB kinases, resulting in phosphorylation of the inhibitory protein IkappaB and its degradation. Degradation of IkappaB frees nuclear factor kappaB in the cytoplasm and allows it to translocate into the nucleus. Following its degradation, IkappaB is rapidly re-synthesized to act as an endogenous inhibitory signal for nuclear factor kappaB. The DNA binding nuclear form of nuclear factor kappaB is usually an heterodimer that includes one 50 kDa (p50) and one 65 kDa (p65) polypeptide. Once within the nucleus, nuclear factor kappaB binds to its consensus sequence on target genes that promote transcription of a variety of genes coding for a number of key molecules in inflammation such as IkappaB, immunoreceptors, cytokines, chemokines, and the inducible forms of cyclo-oxygenase and nitric oxide synthase.

The first studies on the neuroanatomical distribution of IL-1 receptors used radio labelled ligands. Equilibrium binding of recombinant human ^{125}I-IL-1α in brain membrane homogenates and brain slices revealed the presence of binding sites for IL-1 in the mouse but not the rat brain (Ban et al., 1991; Takao et al., 1993). These binding sites are located almost exclusively in the dentate gyrus of the hippocampus and the choroid plexus. They are also found in the anterior pituitary. Using polymerase chain reaction after reverse transcription (RT-PCR), both type I and type II IL-1 receptor mRNAs were found to be expressed in various regions of the mouse brain (Parnet et al., 1994). The disappearance of radioligand binding in the brain of knockout mice for the gene coding for the type I IL-1 receptor is indicative of the predominance of the type I IL-1 receptor in binding radio labelled IL-1 (Bluthe et al., 2000).

In situ hybridization identified the type I IL-1 receptor mRNA in several other structures of the mouse brain than those in which binding of radio labelled IL-1 was observed. These brain structures included the anterior olfactory nucleus, thalamus, hypothalamus, amygdala and cerebellum (Cunningham et al., 1992). Double labelling revealed that the signal for the type I IL-1 receptor was mainly localized in neuronal cells, endothelial cells and epithelial cells of the choroid plexus and ventricles. In the rat brain, in situ hybridization studies confirmed that neuronal expression is mainly concentrated in the hippocampus, but it is also present in a few cell groups in the basolateral nucleus of the amygdala and the basomedian nuclei of the hypothalamus (Ericsson et al., 1995). In contrast to the very limited regional expression of mRNA of the type I IL-1 receptor, mRNA of interleukin-1 receptor accessory protein detected by Rnase protection assay in normal rat brain is expressed at high levels in all brain regions tested (hypothalamus, cortex, hippocampus and cerebellum) (Ilyin et al., 1998).

Immunohistochemistry techniques with monoclonal antibodies directed against the different types of IL-1 receptors in the mouse system have confirmed that both the type I and type II IL-1 receptors are present in the hippocampus, cerebellum and choroid plexus, whereas only the type II IL-1 receptor is found in the paraventricular nucleus of the hypothalamus (French et al., 1999). However, there was no clear evidence of immunolabelling on either vascular endothelial cells or in the meninges. In addition, only limited immunoreactivity was detected on astrocytes of normal adult mouse brain, in contrast to the abundant labelling of reactive astrocytes in mice infected with a neurotropic virus. In the anterior pituitary, both types of IL-1 receptors are present only on those pituitary cells that synthesize and release growth hormone (French et al., 1996; Parnet et al., 1993).

Demonstration of the expression of IL-1 receptors at the protein level in the rat brain has taken longer to be achieved, probably because these receptors are present in very low abundance, under the detection threshold of most neuroanatomical techniques. The first positive neuroanatomical results in favour of the expression of IL-1 receptors in the rat brain were based on the induction of expression of mRNA of the inhibitory protein IkappaB in the brain of rats injected at the periphery with LPS or IL-1 (Laflamme et al., 1999; Quan et al., 1997). Based on the occurrence of a rapid and intense expression of this marker in the endothelium of brain capillaries, parenchymal astrocytes and microglia, these authors proposed that mRNA synthesis of IkappaB can be used as a tool to detect where and how in the brain receptors with a Toll-interleukin-1 receptor domain are activated during peripheral immune stimulation. This strategy has been recently put into question since factors other than nuclear factor kappaB can also regulate IkappaB mRNA synthesis. This is particularly the case of glucocorticoids and noradrenaline of which the levels are increased by proinflammatory cytokines. To circumvent this problem, it is necessary to measure both IkappaB mRNA synthesis and translocation of nuclear factor kappaB. This approach was validated in type I IL-1 receptor knock-out mice and applied to rats injected intraperitoneally with IL-1 (Nadjar et al., 2003). Translocation of nuclear factor kappaB was restricted to endothelial cells of the brain vasculature, epithelial cells of the circumventricular organs, and ependymal cells of the choroid plexus. Astrocytes were also positive in the brain parenchyma, circumventricular organs, and around blood vessels. The use of an antibody raised against the extracellular domain of the type I IL-1 receptor allowed to confirm the expression of the type I IL-1 receptor in venules of the rat brain, but with a higher concentration in certain brain structures including the preoptic area, subfornical organ, supraoptic hypothalamus, followed by the paraventricular hypothalamus, cortex, nucleus of the solitary tract, and ventrolateral medulla (Konsman et al., 2004). No type I IL-1 receptor associated with neurones was found in this study.

It is important to note that the receptors of LPS are also located at the blood–brain barrier interface. Toll-like receptor 4 together with cluster of differentiation 14 (CD14) are critical for the recognition of LPS and activation of the genes for proinflammatory cytokines by a signalling pathway dependent on nuclear factor kappaB. CD14 and Toll-like receptors 4 are constitutively expressed in circumventricular organs, choroid plexus and leptomeninges (Rivest, 2003). Circulating LPS increases the level of expression of CD14 in these structures and in cells lining the brain microvasculature.

4. The peripheral immune message is transmitted to the brain via both neural and humoral communication pathways

The fact that intracerebral administration of IL-1 induces sickness behaviour at much lower doses than those that are active at the periphery appears a priori to be consistent with the possibility that this cytokine that is normally produced by peripheral accessory immune cells needs to enter into the brain to exert its action on brain IL-1 receptors. However, IL-1, like other cytokines, is a relatively big protein of about 150 amino acids that is hydrophilic and therefore is unlikely to cross the blood–brain barrier. For this reason, IL-1 and other cytokines are believed to act on those brain sites that lack a blood–brain barrier and are known as circumventricular organs because of their spatial location close to the brain ventricles. For instance, the organum vasculosum of the lamina terminalis has long been considered as the site of action of peripherally released cytokines, based on results of lesion and knife cut experiments (Blatteis, 2000). Blood-borne cytokines are assumed to diffuse into the brain side of the blood–brain barrier through fenestrated capillaries, and act on brain parenchymal astrocytes to induce the synthesis and release of secondary mediators such as nitric oxide and prostaglandins of the E2 series. These secondary mediators would then freely diffuse to nearby target brain areas, such as the paraventricular nucleus of the hypothalamus and the median preoptic area of the hypothalamus. This mode of action has been proposed to mediate both the corticotropic and pyrogenic effects of cytokines (Katsuura et al., 1990; Stitt, 1985).

The problem with this hypothesis is that it does not account for the fact that cytokines do not act as hormones but as paracrine or autocrine signals within the cellular environment in which they are released. Furthermore, this hypothesis cannot be reconciled with the unexpected observation that cytokines are expressed in the brain in response to peripheral immune stimuli. Expression of cytokines at the periphery by activated accessory immune cells has been found to be consistently associated with a delayed induction of cytokines in the brain. For instance, mice injected intraperitoneally with LPS showed an enhanced expression of IL-1β and TNFα mRNAs in the hypothalamus with a peak at 1 h post-injection, whereas IL-6 and the IL-1 receptor antagonist mRNAs were expressed at a later time, 2–6 h post

injection (Laye et al., 1994). The increase in IL-1β mRNA was associated with an enhanced production of the protein since hypothalamic levels of IL-1β peaked at 4 h post-injection. Like at the periphery, brain IL-1β was responsible for its own production and the production of other cytokines since administration into the lateral ventricle of the brain of the IL-1 receptor antagonist to LPS-treated mice abrogated the expression of IL-1β, TNFα and IL-6 mRNAs in the hypothalamus without altering circulating levels of IL-1β (Laye et al., 2000). Based on immunohistochemistry techniques and consistent with results from in vitro studies, the main cellular sources of IL-1β in the brain were found to be microglial cells and perivascular and meningeal macrophages (van Dam et al., 1992). In contrast to what occurs during brain injury, microglial cells do not need to become activated to produce IL-1 and other cytokines in response to peripheral cytokines. They remain ramified. Most IL-1β-positive cells are detected as macrophages in the meninges, choroid plexus, circumventricular organs, as intermediate cell forms between macrophages and microglia at the surface of the cerebral cortex, in the meninges, and as microglial cells in the hypothalamus (Van Dam et al., 1995). The number of IL-1β-positive microglial cells reaches a maximum 8 h after intraperitonal or intravenous administration of LPS. The temporal pattern of brain IL-1β expression in rats injected intraperitoneally with LPS occurs in two successive waves that have been monitored using immunoreactive c-Fos expression as a marker of the cellular activation that is induced by peripheral immune stimulation (Konsman et al., 1999). The first phase that occurs during the first 2–4 h after LPS injection involves perivascular phagocytic cells in the circumventricular organs and choroid plexus. IL-1β expression in these structures is accompanied by expression of Fos protein in the projection areas of the vagus nerves, including the nucleus tractus solitarius, medial preoptic area, suparoptic nucleus, and central amygdala. From there, the IL-1β immunoreactive signal gradually spreads into the brain parenchyma where it recruits adjacent microglial cells. This results in a second phase of Fos expression in non-neuronal cells in the circumventricular organs and in neurones of the nucleus tractus solitarius and its projection structures. This pattern of diffusion that can be relayed by intermediate molecules such as prostaglandins and nitric oxide obeys the rule of volume transmission. It occurs along a gradient of concentration and follows the blood vasculature and neural bundles. A similar pattern of diffusion has been evidenced using IkappaB mRNA as a marker of IL-1 receptor activation (Quan et al., 1997).

There are several reasons to propose that these locally produced cytokines are responsible for the behavioural effects of peripherally released cytokines. The most obvious one is the fact that much lower doses of IL-1β or TNF-α are needed, when injected directly into the lateral ventricle of the brain or specific brain structures, to depress feeding behaviour and social exploration than the doses which are required when the same cytokine is injected at the periphery (Kent et al., 1992b). The ratio is usually 1 to 100 or 1000. Another finding favouring the possibility of a central site of action of cytokines is the demonstration of the blockade of the behavioural effects of peripherally injected IL-1β by a central injection of the specific antagonist of IL-1 receptors (Kent et al., 1992a). In this experiment, rats were pretreated with the IL-1 receptor antagonist injected into the lateral ventricle of the brain at a dose sufficient to block the depressing effects of IL-1β injected via the same route on social exploration and food-motivated behaviour. This pretreatment completely abrogated the reduction of social exploration induced by intraperitoneal administration of IL-1β and attenuated the decrease in response rate that occurred in IL-1β-treated rats trained to get their food by pressing a lever in a Skinner box.

The existence of an inducible brain cytokine compartment calls for the question of the nature of the communication pathways from the periphery to the brain. Saturable transport of cytokines across the blood–brain barrier is a possible mechanism of communication (Banks et al., 2002). Another possibility is the production of proinflammatory cytokines by perivascular macrophage-like cells in the circumventricular organs in response to circulating pathogen-associated molecular patterns and/or cytokines. These locally produced cytokines would act directly or indirectly on neurones that project to other brain structures or propagate into the adjacent brain parenchyma by volume diffusion, recruiting other cytokine-producing cells “en passant” (Konsman et al., 2000b; Vitkovic et al., 2000). A third and non-exclusive possibility is the transmission of the peripheral immune message to the brain via neural afferent pathways (Dantzer et al., 2000). In accordance with this hypothesis, intraperitoneal administration of LPS was found to increase the levels of sensory neuropeptides (substance P, neurokinin A and calcitonin-gene-related peptide) in the spinal cord (Bret-Dibat et al., 1994) and induce the expression of the cellular immediate-early gene *c-fos* in various areas of the brain (Wan et al., 1993). Inducible expression of the *c-fos* gene has been validated as a widely applicable marker for neural systems activated by a variety of extracellular stimuli. The protein product c-Fos of this gene interacts with nuclear proteins to act as a transcription factor. Fos immunoreactive neurones were identified in the primary projection area of the afferent branches of the vagus nerves, represented by the nucleus tractus solitarius, and secondary projection areas such as the parabrachial nucleus, the paraventricular nucleus and the supraoptic nucleus of the hypothalamus. Furthermore, transection of the vagus nerve at the subdiaphragmatic level, which eliminates afferent neurons originating from the liver and the gastro-intestinal tract, abrogated LPS-induced Fos immunoreactivity in these brain areas (Wan et al., 1994).

The functional nature of this vagal communication pathway between the immune system and the brain was evidenced by the demonstration that section of the vagus nerve abrogated LPS-induced hyperalgesia in rats (Watkins et al., 1994a) as well as LPS- and IL-1β-induced decreases

in social exploration (Bluthe et al., 1994) and food-motivated behaviour (Bret-Dibat et al., 1995) in rats and mice. The vagus nerve transmits to the brain not only the signals that are responsible for the behavioural effects of cytokines but also those that are responsible for fever and pituitary-adrenal activation, since vagotomy has been shown to block these two last responses to LPS (Fleshner et al., 1995; Watkins et al., 1995). In accordance with such a wide range of effects of vagotomy on the brain actions of peripheral immune stimuli, this surgical procedure was found to result in the abrogation of the induction of IL-1β in the brain at the mRNA and protein levels in response to peripherally injected LPS (Laye et al., 1995).

Although all these findings are strikingly suggestive of a neural pathway mediating the effects of peripheral cytokines in the brain, other interpretations are possible. In particular, section of the vagus nerve might alter in a non-specific way the sensitivity of circumventricular organs to circulating cytokines because of the close anatomo-functional relationships between one of these circumventricular organs, the area postrema, and the primary projection area of the vagus nerve, the nucleus tractus solitarius. Another possibility is that the neural reorganization which takes place in the projection areas of the vagus nerve decreases the sensitivity of brain cell targets to cytokines. If the consequences of vagotomy on the behavioural effects of cytokines are due to such mechanisms, vagotomized animals should be less sensitive to the effects of cytokines that are administered by other routes of injection than the intraperitoneal route. However, section of the vagus nerve did not attenuate the behavioural effects of IL-1β when this cytokine was injected intravenously or subcutaneously (Bluthe et al., 1996b) or directly into the lateral cerebral ventricle of the brain (Bluthe et al., 1996a). These results are important because they confirm the role of vagal afferent nerves in the transmission of the immune message from the periphery to the brain and they show that the vagus nerve conveys specific information concerning cytokines injected into the abdominal cavity. Inflammation taking place in other parts of the body is relayed to the brain by other afferent nerves, the glossopharyngeal nerve for instance in the case of the oral activity (Romeo et al., 2001). Although most of the evidence in favour of the role of afferent nerves in the transmission of the peripheral immune message to the brain is based on nerve section studies, other experiments have confirmed such a role using less drastic intervention techniques. Consistent with the observation that most of the afferent vagal fibres are glutamatergic, administration of a glutamate receptor antagonist was found to abrogate the induction of Fos in primary and secondary vagal projection areas of the brain (Wan et al., 1994). In the same manner, reversible inactivation of the dorsal vagal complex of the brainstem in which afferent vagal nerves terminate by a local injection of bupivacaine, a potent local anesthetic agent, totally blocked the induction of Fos in secondary brain areas of the vagus nerves and the decrease in social exploration induced by an intraperitoneal injection of LPS (Marvel et al., 2004).

The existence of several communication pathways from the periphery to the brain calls for the question of their possible complementarity or redundance. This issue has not yet been adequately addressed despite the fact that there is already evidence for a differential role of the various communication pathways. For instance, vagal sensory pathways have been found to be more important for mediating cytokine-induced sickness behaviour than fever or activation of the hypothalamic-pituitary-adrenal axis, since a subdiaphragmatic section of the vagus nerves that abrogated the depressing effects of intraperitoneal LPS on social behaviour had no effect on the pyrogenic and corticotropic effects of this treatment in the same animals (Konsman et al., 2000a). Obviously, a key factor for the predominance of the neural pathway over the humoral pathway is the relative distance of the brain target neural structures from the brain sources of cytokines. In particular, the hypothalamic ventro-medial preoptic area that is involved in the regulation of body temperature can be more easily reached from the organum vasculosum of the lamina terminalis than the extended amygdale that is certainly responsible for social withdrawal. In addition, there is the possibility that peripheral immune activation of afferent sensory nerves sensitizes brain target areas to the action of cytokines and other inflammatory mediators produced in the circumventricular organs and brain venules (Dantzer et al., 2000).

5. Cytokines play a role in learning and memory

The relative abundance of IL-1 receptors in the dentate gyrus of the hippocampus points to a possible role of this cytokine in hippocampal functions including learning and memory. This possibility has been first assessed in the context of cytokine-induced sickness behaviour, by injecting relatively high doses of LPS or IL-1 at the periphery or into the lateral ventricle of the brain. The results show in general an impairing effect of IL-1 on hippocampus-dependent behavioural performance. For instance, rats injected intraperitoneally with LPS were deficient in learning an autoshaped lever pressing task in which they had to associate the protrusion of a retractable lever in a modified Skinner box with the delivery of a food pellet (Aubert et al., 1995b). This effect was not related to IL-1-induced anorexia since IL-1 treated rats still ate the delivered food pellets but were less inclined to press on the lever once it was inserted into the Skinner box. Other instances of the impairing effects of IL-1 on hippocampal-dependent forms of memory have been evidenced in a water maze task with a hidden platform (Gibertini et al., 1995) and in a test of context-dependent conditioned fear (Rachal Pugh et al., 2001). In accordance with these behavioural effects, IL-1 was found to impair long

term potentiation in the hippocampus (Cunningham et al., 1996; Murray and Lynch, 1998).

It is important to note that these findings do not imply that IL-1 impairs all forms of learning. It has been amply demonstrated that IL-1 treated rats and mice can form potent conditioned taste aversions based on the pairing of a new taste solution or a specific spatial location with the sickness this cytokine induces (Mormede et al., 2003; Tazi et al., 1988). Furthermore, low doses of IL-1 that do not impair locomotor activity can facilitate lever press avoidance performance in rats (Brennan et al., 2003). Despite the fact that these last results could actually be accounted for by a decreased pain threshold in IL-1-treated animals, there is evidence that endogenous IL-1 plays a physiological role in learning. In an elegant series of studies carried out in rats, Schneider et al. (1998) showed that long term potentiation in the hippocampus is associated with a long lasting increase in IL-1β gene expression, and that the specific antagonist of IL-1 receptors impairs the maintenance of long term potentiation. Similar experiments by another group failed, however, to demonstrate any change in IL-1β mRNA in response to long term potentiation besides those due to the slice preparation (Jankowsky et al., 2000). In an in vivo preparation selected so as to minimize the amount of injury to the brain, only IL-6 mRNA was found to be upregulated by induction of long term potentiation. This response occurred near the site of stimulation and was detected in non-neuronal cells including astrocytes and probably perivascular macrophages.

As a follow up of these pioneering studies on a possible role of cytokines in learning and memory processes, a number of studies assessed the learning abilities of laboratory rodents in which IL-1 signalling was temporarily or permanently impaired. Administration of the IL-1 receptor antagonist immediately after the first training session impaired memory in a water maze and passive avoidance paradigms (Yirmiya et al., 2002). Furthermore, overexpression of IL-1 receptor antagonist in astrocytes of transgenic mice was associated with the selective impairment of performance in a context-dependent fear task but not in a context-independent fear task in which mice had to respond to a tone previously paired with a painful electric shock. This difference between the two tasks is important since it can be interpreted to suggest that the impairment in learning observed in IL-1 receptor antagonist transgenic mice was probably not related to a deficit in behavioural performance, caused for instance by an altered sensitivity to electric shock. A similar dissociation between these two forms of learning was observed in mice treated chronically with IL-1 receptor antagonist either during development or at adulthood. Results obtained in type I IL-1 receptor knocked-out mice go in the same direction (Avital et al., 2003).

There is still some uncertainty as to the mechanisms that mediate the effects of endogenous IL-1 on memory. The constitutive expression of IL-1β and IL-1 receptors in the hippocampus together with the observation that IL-1 modulated hippocampal long term potentiation do not necessarily imply that the effects observed in vivo are mediated by a direct effect of endogenous IL-1 on hippocampal IL-1 receptors. The improving effects of IL-1 on conditioned fear and on learning of a spatial food location task were found to be blocked by administration of the glucocorticoid receptor antagonist RU486 (Song et al., 2003; Song et al., 2004). Together with the observation that IL-1 receptor antagonist transgenic mice and type I IL-1 receptor knock-out mice display an attenuated response to the removal of the negative feedback of glucocorticoids on the hypothalamic-pitutary-adrenal axis following adrenalectomy (Goshen et al., 2003), it is possible that the memory deficit observed in these animals is a reflection of an impaired response of the pituitary-adrenal axis to the task rather than a direct consequence of the lack of IL-1 signalling. This interpretation is consistent with the well known facilitating effects of pituitary-adrenal hormones on memory (McGaugh and Roozendaal, 2002).

6. The behavioural effects of cytokines are tightly regulated

Sickness behaviour is an adaptive response of the host to an infection, in the same manner than fear is an adaptive response of an organism to a potential danger. The behavioural activity of cytokines therefore needs to be tightly regulated in terms of time of occurrence, intensity and duration. Cytokines have been metaphorically qualified of two-edge swords, in the sense that their beneficial action on the resistance of the host to infection can be surpassed by their deleterious effects when they are expressed inappropriately. There is plenty of evidence in favour of an active participation of proinflammatory cytokines in the neural death mechanisms, astrogliosis and demyelination that occur during various forms of immune and non-immune brain insults (Allan and Rothwell, 2003). A still limited number of studies point also to the possibility that brain overexpression of proinflammatory cytokines early in development leads to profound disturbances in sensory-motor coordination, affect and cognition (Patterson, 2002). Since the occurrence of sickness behaviour is nothing else than the outward expression of a reversible episode of neuroinflammation, it is important that this episode remains localized and non toxic. Several molecular mechanisms contribute to the tight regulation of the neuroinflammation episode. The responsible molecules have been named “endogenous antipyrogens” or “cryogens” (Kluger, 1991), since they have been originally identified on the basis of their ability to oppose the pyrogenic effects of cytokines. Several classes of molecules play such a role, from molecules belonging to the cytokine network (the so-called anti-inflammatory cytokines) to steroid hormones and neuropeptides.

Anti-inflammatory cytokines are potent regulators of the production and actions of cytokines in the central nervous

system. The specific IL-1 receptor antagonist is produced in the brain by the same cells that express IL-1 in response to peripheral activation of the innate immune system (Palin et al., 2001), and its relative ratio to IL-1β determines the extent of neuroinflammation and its functional consequences on memory (Palin et al., 2004). IL-10 injected into the lateral ventricle of the brain abrogates the induction of expression of IL-1 and TNFα whereas intracerebroventricular administration of an anti-IL-10 antibody has the opposite action (Di Santo et al., 1995). The same treatment attenuated the social withdrawal induced by intracerebroventricular administration of LPS (Bluthe et al., 1999). IL-10 also blocks the inhibitory effect of IL-1 on long term potentiation (Kelly et al., 2001). The effects of other anti-inflammatory cytokines, including interleukin-4 and interleukin-13, are more complex since depending on the time of administration of the anti-inflammatory cytokine in relation to the induction of sickness behaviour, the effect can be either protective or synergistic (Bluthe et al., 2001; Bluthe et al., 2002).

Besides its growth factor properties, insulin-like growth factor-1 (IGF-1) can have anti-inflammatory properties, as shown by the ability of this molecule to improve the course of a number of immune-mediated neuropathologies (Dore et al., 1997). In accordance with its putative anti-inflammatory properties, IGF-1 injected into the lateral ventricle of the brain attenuated the development of sickness behaviour in response to LPS injected via the same route (Dantzer et al., 1999). These protecting effects of IGF-1 on the actions of cytokines in the brain appear to be the mirror image of the impairing effects of proinflammatory cytokines on IGF-1 signalling in the brain (Venters et al., 2001).

The potent activating effects of proinflammatory cytokines on pituitary-adrenal activity have already been mentioned. Glucocorticoids represent another class of key molecules in the regulation of sickness behaviour. Adrenalectomy is accompanied by an increased sensitivity to the depressing effects of IL-1β and LPS on social exploration. This effect was mimicked by acute administration of the antiglucorticoid antagonist RU-38486 to intact mice and it was abrogated by the implantation of a corticosterone pellet in adrenalectomized mice (Goujon et al., 1995b). Adrenalectomized animals implanted with a corticosterone pellet have constant levels of corticosterone but are unable to respond to administration of IL-1 by enhanced pituitary-adrenal activity. This phasic response to IL-1 appears to be important in regulating the behavioural effects of cytokines since the protection offered by a corticosterone pellet which ensured plasma levels of corticosterone intermediate between normal and stress levels was effective only against low but not high doses of IL-1β (Goujon et al., 1995a). The mechanism underlying these effects is represented by the down regulation of glucocorticoids on the synthesis and release of cytokines by both peripheral immune cells and brain cells and by the up regulation of the type II IL-1 receptor which acts as a decoy target (Goujon et al., 1996).

The neuropeptide vasopressin is another important regulator of the effects of cytokines in the brain. The role of this neuropeptide in the regulation of fever has been extensively studied (Pittman et al., 1998). The vasopressinergic neurones that are involved in this effect have their cell bodies in the bed nucleus of the stria terminalis and the terminals project to the lateral septum. This vasopressinergic pathway is highly sensitive to circulating androgens in rodents. Castration leads to a dramatic decrease in the content of vasopressin mRNA in the BNST neuronal cell bodies and to a reduction in immunoreactive vasopressin in the terminal areas of the septum. This androgen-dependent pathway is also involved in the regulation of cytokine-induced sickness behaviour (Dantzer and Bluthe, 1992). Central administration of vasopressin attenuated the depressing effects of centrally injected IL-1β on social exploration. Conversely, central injection of an antagonist of vasopressin receptors, which has no biological activity on its own but prevents endogenously released vasopressin to reach its receptors, sensitized rats to the behavioural effects of IL-1 (Dantzer et al., 1991). These last results are important since they suggest that endogenous vasopressin modulates the behavioural effects of IL-1. To determine whether this phenomenon is mediated by an androgen-dependent or independent vasopressinergic pathway, castrated male rats were compared to intact male rats in their sensitivity to the vasopressin receptor antagonist (Dantzer et al., 1991). Castration by itself potentiated the depressing effects of IL-1β on social exploration. Central administration of vasopressin was more effective in attenuating the behavioural effects of IL-1 in castrated than in intact male rats and, conversely, central administration of the vasopressin receptor antagonist was no longer active in potentiating the behavioural effects of IL-1 in castrated male rats lacking vasopressinergic innervation of the lateral septum. The mechanisms by which the brain vasopressinergic system is activated by cytokines and the way in which vasopressin interacts with the effect of cytokines on their target cells are not yet fully understood although recent findings indicate that IL-1 activates vasopressinergic neurones via a nitric oxyde (NO)-mediated inhibition of gamma-aminobutyric acid (GABA) afferents (Ferri and Ferguson, 2003).

7. Conclusions and perspectives

The demonstration of the existence of a brain cytokine system that reorganizes perception and actions of the host in response to activation of the peripheral innate immune system represents a major finding gained from studies carried out in this emerging new interdisciplinary field that is represented by psychoneuroimmunology (Dantzer, 2001a,b; Dantzer, 2004). It has important implications in pathophysiology and therapeutics. Activation of this system in a non-compromised individual allows the organism to respond in a coordinated way to infectious pathogens.

However, this system can also be solicited inadvertently and its activation in an otherwise vulnerable individual can have deleterious consequences. There is for instance experimental and epidemiological evidence that the activation of this system by an episode of systemic infection in subjects suffering from chronic brain inflammation associated with chronic neurodegenerative disease can accelerate neural death and precipitate cognitive alterations (Holmes et al., 2003; Perry et al., 2003). There is growing suspicion that the repeated activation of this system by the cytokines released by cancerous cells or induced by chemotherapy and radiotherapy are responsible for the non specific symptoms of cancer including pain, cachexia, fatigue, sleep disorders, and cognitive alterations (Cleeland et al., 2003). Last but not the least, the activation of this system is certainly responsible for the increased prevalence of mood disorders that is observed in patients suffering from chronic inflammatory disorders including autoimmune diseases, coronary heart disease, and asthma (Capuron and Dantzer, 2003). The alleviation of the symptom burden experienced by patients suffering from these various pathological processes will not be possible without the development of new drugs targeting the mechanisms of production and/or action of proinflammatory cytokines in the central nervous system.

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