The role of interleukin-6 signaling in nervous tissue

Michelle Rothaug, Christoph Becker-Pauly, Stefan Rose-John

Institute of Biochemistry, Christian-Albrechts-Universität zu Kiel, Olshausenstrasse 40, 24098 Kiel, Germany

Abstract

The cytokine interleukin-6 (IL-6) plays a critical role in the pathogenesis of inflammatory disorders and in the physiological homeostasis of neural tissue. Profound neuropathological changes, such as multiple sclerosis (MS), Parkinson’s and Alzheimer’s disease are associated with increased IL-6 expression in brain. Increased nocturnal concentrations of serum IL-6 are found in patients with impaired sleep whereas IL-6-deficient mice spend more time in rapid eye movement sleep associated with dreaming. IL-6 is crucial in the differentiation of oligodendrocytes, regeneration of peripheral nerves and acts as a neurotrophic factor. It exerts its cellular effects through two distinct pathways which include the anti-inflammatory pathway involving the membrane-bound IL-6 receptor (IL-6R) expressed on selective cells, including microglia, and the inflammatory pathway involving the soluble form of the IL-6R/IL-6 complex. Thus, trans-signaling is blocked but classical signaling is completely unaffected. A modified, recombinant dimerized version of sgp130 (sgp130Fc) has successfully been used to block inflammatory processes in mice and may also be used in the clarification of IL-6 trans-signaling in neurological diseases.

1. Introduction

Cytokines are proteins secreted by various cell types, which signal through specific receptors in the regulation of the intensity and duration of the immune response as well as acting as mediators in cellular communication. Some cytokines play a role in signaling within the central nervous system (CNS) in the regulation of sleep, the generation and recall of long-term memory, the focus of attention as well as in neurodegenerative diseases and the integrity of the blood–brain-barrier [1,2]. In this review we focus on the role of IL-6 classical and trans-signaling in nervous tissue, its impact on the onset and progression of neurological disorders, and on potential therapeutic strategies to specifically block either single or both signaling pathways.

2. The pleiotropic nature of interleukin-6

In the mid-eighties several groups reported on cytokines important for the stimulation of B-cells (B-cell stimulatory factor type-2, BSF-2) [3], the growth of virus-infected B cells (interferon-β-2, INF-β-2) [4], regulation of fibrinogen expression in hepatocytes (hepatocyte-stimulating factor, HSF-2) [5] and as a factor released from fibroblasts upon a viral stimulus (the 26-kDa protein) [6]. Almost simultaneous molecular cloning of the cDNA for BSF-2 [7], INF-β-2 [4], HSF-2 [8] and the 26-kDa protein [9] revealed that they represent one identical molecule, now referred to as interleukin-6 (IL-6) [10]. Since then, IL-6 was identified to be involved in tissue regeneration [11–14], inflammation [15–17] and pathogen defense [18]. However, IL-6 signaling is also a major trigger for the onset and progression of many pathological conditions, such as rheumatoid arthritis [19,20], inflammatory bowel disease [21] or sepsis [22], and is therefore a chief focus of pharmaceutical research [23].

IL-6 belongs to a group of four-helical bundle cytokines that includes IL-11, IL-27, leukemia inhibitory factor (LIF), oncostatin M (OSM), cardiotrophin-1 (CT-1), neuropoietin and cardiophrin-like cytokine factor-1 (also known as new neurotrophin 1 and B cell stimulatory factor-3) [24–28]. A cytokine may bind to its corresponding non-signaling α-receptor. This receptor/cytokine complex in turn binds to a β-receptor that sequentially creates a signal. Consequently, cytokines can also be categorized into families based on their target β-receptors. Thus, IL-6 and other cytokines, such as OSM, LIF IL-11 and CT-1, have been grouped into one family as, when in complex with their corresponding α-receptor, they bind a common signal-transducing β-receptor glycoprotein 130 (gp130) [29,30].
IL-6 is a potent inducer of the hepatic acute phase response [31], is involved in lymphocyte and monocyte differentiation and acts on B-cells, T-cells, hepatocytes and hematopoietic progenitor cells [32]. It also plays a critical role in the normal homeostasis of neuronal tissue as its absence leads to reduced glial activation in traumatic brain injury as well as changes in sleeping behavior [33–35]. On the other hand, IL-6 overproduction in the brain leads to neurodegeneration [36,37].

IL-6 can be secreted by both immune (T-cells, B-cells, macrophages and microglia) [38–41] and non-immune cells (muscle cells, adipocytes, fibroblasts, endothelial cells, neurons) [32,42–45]. In contrast, the IL-6 receptor (IL-6R) is only found on a restricted subset of cell types, including hepatocytes [46], some leukocytes [47] and microglia [48,49] but not on oligodendrocytes [50] or astrocytes [48]. However, the IL-6R also exists as a soluble protein (sIL-6R) either generated via alternative splicing [51,52] or limited proteolysis [53] by metalloproteases [54] including A Disintegrin And Metalloproteinase (ADAM) family members ADAM10 and ADAM17 [55,56] (Fig. 1a). The IL-6R lacks intrinsic signal transduction capacity. Therefore, signaling is induced through the binding of the IL-6/IL-6R complex to its β-receptor gp130, which prevents unwanted, non-specific cellular activation (Fig. 1). In order for the binding of this complex to elicit a cellular response, through the phosphorylation of downstream targets such as JAK/STAT, ERK and PI3 kinase, gp130 must be present at the cell surface [57]. Thus, regulation of signaling is mediated by the availability of the sIL-6R.

3. IL-6 classical versus trans-signaling

IL-6 may stimulate a response in a target cell in two different manners. Classical signaling involves the binding of IL-6 to the membrane-bound IL-6R which initiates dimerization of gp130 and subsequent downstream signaling [58,59] (Fig. 1a). Alternatively, the sIL-6R may form a complex with IL-6 and stimulate distant cells that express gp130 but not surface-bound IL-6R, including most neuronal types, endothelial cells, oligodendrocytes and many other cell types, in a process that is termed trans-signaling [60] (Fig. 1b). Such cells, in the absence of the sIL-6R, would not be able to respond to IL-6. In contrast to the expression of the IL-6R, gp130 is ubiquitously expressed on almost all cell types [61] including astroglial cells [62] and neurons [42]. As we know now, the majority of cells that respond to IL-6 during inflammation do not express the IL-6R and are thus not directly responsive to the cytokine. Therefore, trans-signaling allows cells distal to the source of sIL-6R release to be targeted. As mentioned above, classical signaling is critical for anti-inflammatory signals in contrast to pro-inflammatory trans-signaling. IL-6 trans-signaling differs from classic signaling in its kinetics, often leading to fundamentally different cellular responses [63]. Hepatocytes seem to require IL-6 trans-signaling for tissue regeneration after acute injury [11,12,64]. However, IL-6-mediated neurodegeneration [65], inflammatory colon cancer [15], arthritis [66,67] and inflammatory bowel disease [21,68] are mediated by trans-signaling. Interestingly, a soluble differentially spliced form of gp130 is expressed under normal physiological conditions and is capable of binding the complex of sIL-6R/IL-6 and thus selectively blocking trans-signaling [69,70]. This provides the basis for a therapeutic strategy to block trans-signaling-dependent pro-inflammatory conditions described in Section 7 under “therapeutic targets”.

4. Targets of IL-6 signaling in the nervous system

Low levels of IL-6 are present in brain under physiological conditions. A dramatic increase in expression and secretion of IL-6 is observed during various neurological disorders including Alzheimer’s (AD) and Parkinson’s disease (PD) [71], brain cancer [72], multiple sclerosis (MS) [73,74] and brain ischemia [75]. Lack of IL-6 signaling plays a critical role in inflammation as well as neuroprotection, demonstrated by decreased glial activation in IL-6-deficient mice in response to traumatic CNS injury [33,35]. However, IL-6 also promotes the differentiation of oligodendrocytes [76–78], acts as a neurotrophic factor [79–82] and is important in the regeneration of peripheral nerves [83]. These functions highlight the pro-survival effect of IL-6 in CNS pathology.

Fig. 1. Overview of IL-6 classical and trans-signaling. (A) In classical signaling, binding of IL-6 to the membrane-bound α-receptor IL-6R causes the dimerization of its β-receptor gp130 which leads to downstream JAK/STAT signaling in a restricted subset of cells. Shedding of the IL-6R by the ADAMs leads to the liberation of sIL-6R which can bind free IL-6. (B) This complex may then elicit JAK/STAT signaling in distal cells that express gp130 but lack the membrane-bound IL-6R in a process termed trans-signaling.
Interestingly, IL-6-mediated neuronal degeneration [37] depends on trans-signaling in the CNS [65] whereas classical signaling has a regenerative role in neural tissue. This emphasizes the importance of distinguishing between the two pathways when designing drugs for the treatment of neurological diseases. IL-6R mRNA was detected in cultured microglia, astrocytes and neurons [50]. However, IL-6R protein could only be detected in microglial cells [48]. This may be due to the production of the spliced version of the IL-6R that gives rise to soluble receptor [51] and would correlate with reports that sIL-6R transcripts can be detected in human brain [84]. Thus, astrocytes and neurons appear to exclusively express the sIL-6R, which is excreted from the cell and is therefore undetectable in cell lysates. Although IL-6 alone can elicit gp130-mediated signaling in microglial cells, they are also responsive to hyper-IL-6 (a recombinant chimera of IL-6 and the sIL-6R that molecularly mimics trans-signaling) [48,85]. Importantly, classical signaling does not appear to cause the activation of microglial cells in pathological states as they only become pro-inflammatory when exposed to hyper-IL-6 when compared to IL-6 alone [49] highlighting the functional differences between classical and trans-signaling. In fact, classical signaling via IL-6 is hypothesized to have a protective role within the CNS [86] by accelerating nerve regeneration following trauma [83] as well as being neuroprotective following spinal cord injury [87].

5. Neurodegenerative diseases

5.1. Geriatric neurodegenerative diseases

IL-6 is generally altered in the CNS where neuro-inflammation appears to play a role in neurodegenerative disorders such as PD [88,89] and AD [90–92], the most common neurodegenerative disorders in an ageing population. Both conditions are caused by the accumulation of what are believed to be neurotoxic proteins leading ultimately to neuronal death [93,94]. AD is characterized by progressive loss of memory finally culminating in dementia. Neuropathologically, it is described as the accumulation of the β-amyloid peptide (Aβ) (a proteolytic cleavage product of the amyloid precursor protein (APP)) as well as intraneuronal inclusion of phosphorylated Tau [95] where the hippocampus and cortical regions are particularly affected. Both environmental and genetic risk factors play a role in its presentation [96,97]. To date, no treatment is available to prevent the progression of disease.

Aβ can activate glial cells in culture thereby inducing the production of a variety of inflammatory products including IL-6 [98,99]. In fact, intracerebroventricular (ICV) administration of Aβ is enough to elevate IL-6 levels in the periphery [100]. Interestingly, the transcription of APP is stimulated via IL-6 trans-signaling [101]. Furthermore, the addition of IL-6 to culture media significantly increased neurotoxicity caused by Aβ in cortical neurons [102]. However, despite the clear synergistic effect of IL-6 expression and Aβ toxicity shown in vitro, the cytokine was shown to be beneficial early in the disease process by potentially enhancing plaque clearance through the activation of astrocytes and microglia in vivo [103].

Similarly, IL-6 was previously shown to be neurotrophic [104] in pure cultures of dopaminergic neurons as well as protective against neurotoxic effects elicited by 1-methyl-4-phenylpyridinium (MPP⁺), a compound used to mimic the selective neuronal loss observed in PD [79]. In this case signaling is probably mediated via secreted sIL-6R in the media, as discussed in Section 3. PD is pathologically characterized by the loss of dopaminergic motor neurons within the substantia nigra as well as the intraneuronal accumulation of α-synuclein [105]. IL-6-deficient mice were significantly more susceptible to neuronal death in a mouse model where MPP⁺ was also used [106], highlighting the importance of IL-6 signaling for the maintenance of healthy neurons. Further studies are necessary to elucidate the role of trans-signaling in the pathogenicity of PD and AD.

5.2. Multiple sclerosis

MS is an inflammatory autoimmune disease with unknown etiology [107] where instilling myelin sheaths are successively destroyed leading to an array of neuropathological symptoms including pain, mental and sometimes psychiatric problems [108]. To date, treatment consists of alleviation of symptoms as well as physical therapy as there is no known cure for the disease. IL-6 signaling was demonstrated to be absolutely essential for the presentation of disease. This was highlighted in IL-6-deficient mice that are completely resistant to induction of MS-like symptoms in a mouse model of experimental autoimmune encephalomyelitis (EAE) [109–111]. Whereas other knockout rodents have shown reduced scores in EAE [112,113], IL-6-deficiency is one of the few knockout mouse strains that shows defective (T-helper) Th17 differentiation [114,115]. In fact, Th17 responses are also impaired in the absence of IL-6 [116]. Interestingly, IL-6-deficient mice exhibit reduced signs of inflammation at the onset of disease in other autoimmune mouse models such as arthritis [19,20] and irritable bowel syndrome [117] where trans-signaling was blocked.

There are two separate pathological processes that sequentially take place in order for IL-6-dependent EAE symptoms to occur. The initial phase begins in the periphery of the mouse before traversing the blood–brain barrier and entering the CNS (Fig. 2). To establish the contribution of the sources of IL-6 to the development of the disease, Quintana and colleagues analyzed the role of such tissue-localized production of IL-6 in the induction and evolution of EAE, using transgenic mice with astrocyte-targeted production of IL-6 (under the control of a GFAP, glial fibrillary acid protein, promoter) [65]. These GFAP-IL-6 transgenic mice develop neuropathological manifestations including degeneration of the gray and white matter [118]. However, due to strong expression of IL-6 in the cerebellum, ataxia symptoms overshadowed further potential roles of IL-6 in the neuropathology of EAE in other brain regions [37,118]. Additionally, mice expressing IL-6 under the control of a neuron-specific promoter exhibited reactive gliosis as observed in GFAP-IL-6 mice but no neurodegeneration [36] potentially due to dose-dependent effects. GFAP-IL-6 mice have been crossed into the IL-6-deficient background. These GFAP-IL-6/IL-6-deficient mice did not show resistance to EAE as observed in IL-6-deficient mice [119]. Rather, they clearly developed symptoms of neurological disease despite restricted expression of IL-6 in nervous tissue. These findings suggest that production of IL-6 in the brain alone may promote an autoimmune response to a CNS antigen in the absence of peripheral IL-6.

Trans-signaling within the CNS is the prevailing mechanism responsible for IL-6-mediated neuropathology in mice [65]. Mice crossed from the GFAP-IL-6 strain with recently generated transgenic mice dubbed GFAP-sgp130Fc (where IL-6 trans-signaling can be distinguished from classic signaling within the CNS (Fig. 3A)) exhibit almost complete abolishment of neurodegenerative changes showing that trans-signaling in the brain is the destructive force behind IL-6-dependent neurodegeneration observed in the GFAP-IL-6 mice [65]. However, we have observed that GFAP-sgp130Fc mice (Fig. 3A) are completely vulnerable to induction of EAE, comparable to wild-type controls (Fig. 3B,C). GFAP-sgp130Fc mice and wild-type controls were immunized with a myelin oligodendrocyte glycoprotein peptide (MOG35-55) with complete Freund’s adjuvant (CFA) (Hooke laboratories, Lawrence, MA, USA) over a period of 16 days. Disease severity and weight fluctuation was measured for the duration of the experiment. Both genotypes showed the same progression of disease (Fig. 3B) as well as variations in weight (Fig. 3C). Furthermore, an extended experiment where mice were scored for 30 days after immunization showed a similar recovery pattern when comparing both genotypes (data not shown). Therefore, trans-signaling does not play a major role in the pathogenic progression of MS-like symptoms in mice.

Consequently, it is likely that IL-6-dependent signaling (classical and trans-) within the periphery is the main driving force behind the
pathogenesis of MS. In fact, during the pathological course of MS T-cells differentiate into Th17-cells after stimulation with IL-6, in combination with TGF-β, in the periphery. T-cells differentiate into Th17-cells in a TGF-β/IL-6-dependent manner within the periphery (Fig. 2) [116]. IL-6 controls the balance between regulatory T-cells and Th17-cells [120]. In the absence of IL-6, TGF-β alone induces T-cell differentiation into regulatory T-cells, thus promoting an anti-inflammatory rather than a pro-inflammatory response [110]. Furthermore, the induction of Th17-cells by IL-6 is strongly increased by the presence of sIL-6R indicating a role for trans-signaling in this process [121].

Additionally, injection of an anti-IL-6R monoclonal antibody (clone MR16-1, which blocks both classical and trans-signaling) into the peripheral blood of EAE-induced wild-type mice greatly diminished onset and progression of disease when compared to IgG treated control mice [122]. Furthermore, a study by Linker and colleagues used peripheral treatment of mice with recombinant sgp130Fc (thereby effectively blocking trans-signaling outside of the CNS) while concomitantly inducing EAE. Mice treated with peripheral injection of sgp130Fc exhibited a significantly later onset of symptoms [123]. However, in both EAE studies the symptoms were not completely abolished as was seen in IL-6-deficient mice pointing to a potential minor role of trans-signaling in the CNS (Fig. 2).

In the next step of pathogenesis in MS, the blood–brain-barrier integrity is disrupted via IL-6-mediated upregulation of VCAM-1 [111], allowing T-cells to infiltrate the brain. Interestingly, sIL-6R is produced by activated T-cells [124], thus providing a pool of soluble receptor for trans-signaling within the periphery and within the CNS from cells that have crossed the blood–brain barrier. The IL-6R expressed on microglia [48] also provides a potential source of sIL-6R as evidenced from elevated levels of trans-signaling in aged mice that have higher levels of the receptor and its shedding enzyme ADAM17 than their younger counterparts after neurotoxic insult [125]. However, as mentioned earlier, trans-signaling within the brain does not appear to be critical for the progression of disease (Fig. 3B/C). In a further pathogenic step, IL-17, produced by Th17-cells, stimulates IL-6 expression in astrocytes in a positive feedback-loop [126,127]. Trans-signaling is reported to activate astrocytes [128] and contribute further to elevation of IL-6

**Fig. 2.** Overview of the pathogenesis in MS. (1) T-cell response: naïve T-cells are differentiated into Th17-cells after stimulation with IL-6, in combination with TGF-β, in the periphery. During the process of differentiation of naïve T-cells to Th17-cells, sIL-6R is released from the surface. Additionally, trans-signaling (via IL-6 and IL-6R) leads to increased activation of naïve T-cells. (2) Crossing the blood–brain barrier (BBB) via increasing VCAM-1 on endothelial cells: increased levels of IL-6 directly influence the surface expression of the cell adhesion molecule VCAM-1, allowing T-cells to traverse the endothelial layer along with IL-6. (3) T-cell differentiation and secretion of sIL-6R: elevated IL-6 levels leads to differentiation of naïve T-cells that have crossed the BBB. (4) Feed-back activation loop: IL-17 released from Th17-cells leads to activation of astrocytes which release more IL-6 causing a pathological feed-back loop of activated T-cells. (5) Microglial/astrocyte activation: elevated sIL-6R and IL-6 within the CNS also cause the activation of microglia cells and enhance the expression and secretion of IL-6 from astrocytes. (6) Attack of myelin and demyelination of axons: reactive oxygen species (ROS), released from activated astrocytes, in combination with activated microglial and Th17-cells, ultimately leads to demyelination of the axon resulting in descending paralysis. Trans-signaling pathways are highlighted with red arrows. Trans-signaling within the CNS is not critical for progression of disease.
concentrations of sIL-6R in healthy individuals during a regular sleep.

Dimitrov and colleagues measured the plasma concentrations of sIL-6R released from activated astrocytes [130] and microglia [131] also contributed to myelin sheath damage due to their toxic effects on oligodendrocytes [132]. Furthermore, trans-signaling plays a role in the activation of microglia cells that also contribute to the destruction of myelin fibers. In fact, interferon-γ-dependent elevation of CD40 in microglia cells was elevated when primary cultures were exposed to hyper-IL-6 when compared to IL-6 alone [49]. This is in contrast to the proposed neuroprotective properties of microglia shown after stimulation with IL-6 alone in classical signaling [86]. Finally, demyelination leads to ascending paralysis and eventually, after repeated demyelination events, to destruction of the axon causing lesions within the CNS [133,134].

6. Sleep

The immune system, under physiologic and pathophysiologic condition, exerts control over the pattern of sleep [135,136]. It is believed that this control is mediated by cytokines such as TNFα, IL-1β, and IL-6. It is, however, not well understood, whether this control is executed within the CNS or within the periphery. In the case of IL-6 such experiments have been recently conducted and therefore contribute to the understanding of the biology of this cytokine.

Sleep deprivation is associated with reduced antibody titer after immunization [137]. In fact, sleep was shown to modulate the rate of IL-6 trans-signaling. Dimitrov and colleagues measured the plasma concentrations of sIL-6R in healthy individuals during a regular sleep–wake cycle and compared their results to samples taken during 24 h of wakefulness. They clearly showed that the proteolytically cleaved form of the IL-6Rα rather than the differentially spliced form was significantly elevated during sleep [138]. Additionally, the shedding of the IL-6R, ADAM17 is expressed in a sleep-dependent manner, at least in macrophages [139].

In rats, IL-6 augments NREM (non-rapid eye movement sleep, a transitional role in sleep cycling) [140], and more specifically, slow wave activity during SWS (slow-wave sleep which includes later stages of sleep often known as “deep sleep” that is important for cerebral restoration and recovery [141,142]) in humans [143]. Paradoxically, impaired sleep was reported to correlate with elevated nocturnal IL-6 levels in humans [144–146]. In agreement with this, IL-6-deficient mice spend more time in REM (the final stage of sleep associated with dreaming [147]) when compared to control mice [34]. Such studies highlight the cryptic role of IL-6 signaling in sleep. The heterogeneity of results from the observed IL-6-dependent effects on sleep can be partially explained by the fact that IL-6 can act on different cell types via classical or trans-signaling. Alternatively, differences in concentration as well as binding affinity between animal and human models may contribute to variances in results. In fact, some sleep-inducing cytokines have an anti-somnogenic activity at higher concentrations that resembles sleep that occurs during severe infectious disease states.

A previous study showed that an increase in trans-signaling enhanced REM but did not influence NREM in mice after ICV injection of hyper-IL-6 [148]. Interestingly, using the (phosphoenolpyruvate carboxykinase) PEPCK-sgp130Fc [16] and GFAP-sgp130Fc mice (Fig. 3A) that block trans-signaling in the periphery and CNS respectively, it could
be concluded that peripheral and CNS trans-signaling influence sleep in a different manner [16,65]. The mechanism of how sgp130Fc blocks trans-signaling is described in Section 7. In fact, trans-signaling in the periphery plays a more profound role than signaling within the CNS as PEPCK-gp130 transgenic mice spent less time in all stages of sleep (NREM as well as SWS and REM) than their control mice. In contrast, trans-signaling blockage in the CNS led only to a slight decrease in time spent in REM [149]. Thus, therapy that targets trans-signaling in the brain would have very little predictable side-effects in relation to sleep.

7. Therapeutic targets

IL-6 has been shown to play a fundamental role in neurological disorders as discussed in this review. Therefore, it seems reasonable that a blockade of IL-6 signaling could be a treatment option for neuroinflammatory conditions. The majority of IL-6-related treatments focus on preventing inflammation and target total signaling but not specifically trans-signaling (Fig. 4) [23]. Blocking IL-6 with neutralizing anti-IL-6 monoclonal antibodies (B-E4 and B-E8) have been used in clinical trials for the treatment of multiple myeloma with minimal side-effects [150]. However, treatment proved ineffective in the long term due to exaggerated increased levels of circulated IL-6 that override the neutralizing activity of the antibody [151]. As an alternative method to block IL-6 signaling a monoclonal antibody against the IL-6R (Tocilizumab), which binds to the membrane-bound and soluble IL-6R (Fig. 4) thereby blocking binding of IL-6, has been developed. Tocilizumab is approved for the treatment of rheumatoid arthritis as well as systemic and polyarticular juvenile inflammatory arthritis. [152]. The efficiency of this treatment in prevention of inflammation has been successfully shown in patients with juvenile idiopathic arthritis [153,154] as well as in a mouse model of MS [122]. However, as we have already highlighted, a problem with such therapies is that the primary pro-inflammatory nature of trans-signaling as well as the regenerative, anti-pathogenic function of classical signaling are simultaneously targeted leading to a higher propensity for infections in treated patients [154].

Another strategy is based on the capacity of the physiological soluble form of gp130 (sgp130) capable of blocking trans-signaling [69,70]. In fact ICV injection of sgp130 accelerated recovery and reduced neuropathological changes in response to lipopolysaccharide-induced endotoxemia in mice [125]. Furthermore, studies in animal models of stress observed that an IL-6-dependent reduction in prefrontal cortex synaptic inhibition, associated with stress, could be blocked using ICV application of sgp130 [155] and was absent in GFAP-sgp130Fc mice [156]. Another approach to expand on this idea is the generation of a designer protein sgp130Fc with increased affinity for the IL-6R/IL-6 complex. It is a fusion protein of the extracellular part of sgp130 and the constant Fc portion of human IgG1 antibody [70] (Fig. 4), identical to that overexpressed in GFAP-sgp130Fc [65] and PEPCK-sgp130Fc mice. In fact, recombinant gp130Fc has already undergone evaluation in phase I clinical trials [157,158]. This protein specifically binds to the complex of IL-6 and its receptor, but not to IL-6 or the IL-6R alone. Importantly, due to the fact that soluble sgp130Fc selectively inhibits IL-6 trans-signaling, this provides a means to molecularly distinguish between IL-6 classical and trans-signaling. However, with regard to neuropathological conditions where IL-6 trans-signaling may play a role, this protein is not capable of crossing the blood–brain barrier as it is exclusively detected within the CNS of GFAP-sgp130Fc mice (Fig. 3A). Receptors on the blood–brain barrier bind ligands to facilitate their transport, thus, recombinant proteins can be modified in order to mimic this selective process thereby facilitating its entry into the CNS via receptor-mediated transcytosis [159]. Well characterized blood–brain barrier receptors suitable for exploitation in the reengineering of proteins for such a task include the transferrin receptor (TfR) and insulin receptor (IR) [160]. Re-engineered TfR/IR IgG-fusion proteins that are capable of penetrating the CNS and eliciting a therapeutic effect have successfully been used in models of neurological disease [160]. Fusion proteins with the constant region of antibodies directed against the TfR for mice [161,162] or the IR (IrmAb) in humans [163,164] have been shown to traverse the blood–brain barrier and represent promising

Fig. 4. Summary of available therapeutic options for the treatment of IL-6-mediated inflammatory diseases. Current monoclonal antibodies directed against either the IL-6R or IL-6 itself target both classical and trans-signaling, thereby preventing T-cell-mediated inflammation. However, such antibodies also bind to membrane-bound IL-6R as well as the sIL-6R, thereby preventing anti-inflammatory as well as pathogenic defence processes. In contrast sgp130Fc can selectively block trans-signaling leading to a reduction in potential side-effects of treatment.
technology in the development of therapies for neurological disease. In this respect, generation of an IRmAb-sgp130Fc chimera may, in theory, aid in its transport over the blood–brain barrier. Alternatively, addition of a biotin label to the Fc part of sgp130Fc would allow it to be recognized by an avidin tagged IRmAb as described for other proteins. Binding of avidin labelled proteins in this manner has been shown to dramatically increase transport into the CNS [165, 166]. Naturally, further studies are necessary to test the efficiency of such chimeras to penetrate the blood–brain barrier and their ability to block trans-signaling.

8. Concluding remarks

IL-6 is a central player in physiological neuronal and glial function as well as neuroinflammatory pathways observed in diseases of the CNS. The significance of IL-6 signaling is appreciated in a wide range of diseases [19, 21, 72, 74, 167] highlighted by the development of therapies aimed at counteracting this pathway [23, 150, 153, 154]. Due to the critical role of IL-6 in host defense and glial/neuronal homeostasis further studies are necessary to clarify the individual cellular consequences of IL-6 signaling within the CNS. While the pro-inflammatory trans-signaling pathway in the periphery is essential for susceptibility of mice to MS-like symptoms, we have shown that it only plays a minor role within the CNS. Nevertheless, this is probably different for other neurodegenerative diseases, as IL-6 trans-signaling has been shown to cause neuronal damage in mice. Such research is essential to help in the identification of effective therapies and their possible side-effects in the targeting of the grossly pro-inflammatory trans-signaling pathway in neuroinflammatory disorders.

Acknowledgments

The authors would like to thank Dr. Anna Otte, Frederike Schmidt, Dr. Philipp Arnold, Inez Götting, Neele Schumacher, Dr. Katja Möller-Hackbarth, Dr. Ute Pichkinke, Olga Matveeva and Wiona Burmeister for their help with the EAE experiments. This work was supported by Deutsche Forschungsgemeinschaft in SFB 654 (Plasticity and Sleep; project C05) and in SFB 877 (Protelysis as a Regulatory Event in Pathophysiology; projects A1 and A9).

References


T. Skurk, C. Alberti-Huber, C. Herder, H. Hauner, Relationship between adipocyte
I.L. Campbell, C.R. Abraham, E. Masliah, P. Kemper, J.D. Inglis, M.B. Oldstone, L.
M.A. Klein, J.C. Moller, L.L. Jones, H. Bluethmann, G.W. Kreutzberg, G. Raivich,
T. Kishimoto, S. Akira, M. Narazaki, T. Taga, Interleukin-6 family of cytokines and
T. Li, S. He, Induction of IL-6 release from human T cells by PAR-1 and PAR-2 ago-
H.W. Lin, S.W. Levison, Context-dependent IL-6 potentiation of interferon-
M.P. Hsu, R. Frausto, S. Rose-John, I.L. Campbell, Analysis of IL-6/gp130 family re-
C.R. Gomez, J. Goral, L. Ramirez, M. Kopf, E.J. Kovacs, Aberrant acute-phase
T. Taga, T. Kishimoto, Gp130 and the interleukin-6 family of cytokines, Annu. Rev.
T. Li, S. He, Induction of IL-6 release from human T cells by PAR-1 and PAR-2 ago-
M.V. Hobbs, R.J. McEvilly, R.J. Koch, G.J. Cardenas, D.J. Noonan, Interleukin-6 pro-
E.N. Benveniste, Cytokine actions in the central nervous system, Cytokine Growth
B. Dimitriades-Schmutz, S. Rose-John, U. Otten, Role of
M. Hibi, M. Murakami, M. Saito, T. Hirano, T. Taga, T. Kishimoto, Molecular cloning
Y. Kushima, H. Hatanaka, Interleukin-6 and leukemia inhibitory factor promote the
D.L. Gruol, T.E. Nelson, Physiological and pathological roles of interleukin-6 in the
J.A. Wagner, Is IL-6 both a cytokine and a neurotrophic factor? J. Exp. Med. 183
M. Peters, P. Schirmacher, J. Goldschmitt, M. Odenthal, C. Peschel, E. Fattori, G.
P. März, K. Heese, B. Dimitriades-Schmutz, S. Rose-John, U. Otten, Role of interleukin-
M. Penikowa, T. Moos, J. Carrasco, H. Hadberg, A. Molinero, H. Bluethmann, J.
S. Toulmond, X. Vige, D. Fage, J. Benavides, Local infusion of interleukin-6 into the
M. Kopf, E.J. Kovacs, Aberrant acute-phase
T. Kishimoto, S. Akira, M. Narazaki, T. Taga, Interleukin-6 family of cytokines and
eukine-6 gene expression in human endothelial cells: RNA start sites, Int.
S. Matsumoto, S. Rose-John, A. Suzuki, T. Hara, N. Tomiyasu, K.
M. Hibi, M. Murakami, M. Saito, T. Hirano, T. Taga, T. Kishimoto, Molecular cloning
M. Hibi, M. Murakami, M. Saito, T. Hirano, T. Taga, T. Kishimoto, Molecular cloning
T. Taga, T. Kishimoto, gp130 and the interleukin-6 family of cytokines, Annu. Rev.
T. Taga, T. Kishimoto, gp130 and the interleukin-6 family of cytokines, Annu. Rev.


