Exploring the role of interleukin-27 as a regulator of neuronal survival in central nervous system diseases

Interleukin-27 and Its Receptors
Interleukin-27 (IL-27) is a heterodimeric cytokine composed of a 28 kDa IL-27p28 subunit and the 24 kDa Epstein Barr Virus-induced gene 3 (EBI3) protein. IL-27 subunits are induced primarily in antigen-presenting cells after stimulation of Toll-like receptors, CD40, complement, and interferon (IFN) receptors, which positions IL-27 as a central responder to numerous inflammatory signals. Examination of publicly available single-cell RNA sequencing databases from mouse retina and brain indicates that EB13 and IL-27p28 are detected in multiple glial and neuronal cell subtypes (singlecell.broadinstitute.org). Immunodetection and transcript analyses also confirmed IL-27 expression in non-inflammatory cells in the central nervous system (CNS), including spinal cord neurons, retinal ganglion cells, and photoreceptors (Amadi-Obi et al., 2007).

IL-27 belongs to the IL-6/IL-12 cytokine superfamily that shares receptors and signaling pathways with other cytokines, including cytokines and growth factors such as IL-6, LIF, OSM, and CNTF. The expression of IL-27 and its receptor are rapidly induced after neuronal damage. For example, EB13, IL-27p28, and IL-27RA transcripts are up-regulated in inflammatory cells in the CNS at the peak of pathology in an experimental autoimmune encephalomyelitis (EAE) model of multiple sclerosis (MS) (Li et al., 2005), and IL-27 expression is increased during intraocular inflammation (uveitis) (Lee et al., 2011). Although both receptor subunits are typically required for IL-27 signaling, homodimers of IL-27RA are functional in certain cell types, such as transformed hematopoietic cells (Pradhan et al., 2007). Furthermore, IL-27RA interacts with other receptor complexes, including ciliary neurotrophic factor receptor and IL-6Ra, which expands the number of potential cellular pathways stimulated by IL-27. Interestingly, a soluble form of IL-27Ra was identified in CD4+ and CD8+ T cells, B cells, monocytes, human sera, and COS7 cells that binds to and inhibits IL-27 signaling, indicating an additional route of IL-27 regulation (Dietrich et al., 2014), although the conditions that promote its formation are not yet understood.

IL-27 signals through the JAK-STAT pathway, which is a well-described pathway that mediates many cellular processes, including cellular differentiation, proliferation, and immunologic responses (Burrell et al., 2020). JAKs are receptor-associated tyrosine kinases that are activated when bound to ligands including cytokines, growth factors, and interferons. The gp130 component of the IL-27 receptor engages JAK1 or JAK2, and WSX-1 binds to Tyk2, which catalyzes tyrosine phosphorylation and creates a binding site for SH2 domain-containing STAT proteins. STATs are a family of transcription factors with varied tissue expression, promoter binding affinity, and responses to extracellular signaling. STATs are phosphorylated in the cytosol by activated JAKs, leading to STAT dimerization and activation. STAT dimers are then transported to the nucleus where they induce transcription of target genes. The anti-inflammatory and cytoprotective activities of IL-27 have been linked to STAT1 or STAT3 activation in microglia, dendritic cells, and astrocytes (Amadi-Obi et al., 2007; Lee et al., 2011; Luo et al., 2021), described below. The involvement of JAK-STAT pathways in numerous cellular processes in the CNS increases the potential roles for IL-27 throughout the nervous system, suggests points of cross-talk between IL-27 and other extracellular stimuli, and provides additional regulatory pathways that potentially enhance or suppress IL-27 signaling.

Cell Types Mediating the Effects of Interleukin-27
The role of IL-27 in regulating T cell responses that prevent immune hyperactivity has been extensively characterized and IL-27 has been investigated as a possible therapeutic for chronic inflammatory conditions with excessive T cell activation, such as MS and rheumatoid arthritis. IL-27 antagonizes differentiation and function of type 1 effector cells (Th1), type 2 effector cells (Th2), and IL-17 producing helper T cells (Th17), inhibits Th0 cell differentiation and function, and regulates B cell responses. In contrast, IL-27 has pro-inflammatory functions by promoting the clonal expansion of naive CD4+ T-cells, promoting Treg and CD8+ T cell survival, and stimulating tumor-specific cytotoxic T cell responses (Kim et al., 2019). IL-27 also regulates anti-viral responses of CD4+ T-cells by controlling cell number and effector function (Wehrens et al., 2018), and it promotes the expression of inhibitory receptors
Roles of Interleukin-27 in the Central Nervous System

IL-27 increases neuronal survival in the CNS in various diseases and injuries. For example, intraperitoneal injections of IL-27 reduced ischemia-reperfusion injury in a murine stroke model, decreased infarct volume, and improved neurological outcomes in mice (Milan et al., 2018). IL-27 also protected neurons from excitotoxicity by reducing toxicity from glutamate and other excitotoxic agents (Zhou et al., 2017). Further studies using neuron-specific or glia-specific IL-27 knockout mice will be needed to determine the specific roles of IL-27 in the CNS and to develop therapeutic strategies for neurodegenerative diseases.

Molecular Mechanisms of Interleukin-27-Induced Cytoprotection

IL-10

Studies in animal models of disease and cultured cells demonstrated that multiple signaling pathways contribute to the anti-inflammatory effects of IL-27. In addition to the effects on Th17 cells, IL-27 induces secretion of the anti-inflammatory cytokine IL-10 from macrophages and activated CD4+ T cells (Forseca et al., 2019). In the CNS, IL-27 also promotes anti-inflammatory and regulatory T cell responses, which contribute to the protective effects of IL-27 in neurodegenerative disease. For example, IL-27 reduced apoptosis by acting directly on cultured neurons (Luo et al., 2021). Additionally, Lee et al. (2011) demonstrated that retinal ganglion cells and photoreceptors express basal levels of IL-27 and that IL-27 expression is upregulated in these neurons and microglia in a mouse uveitis model. Furthermore, photoreceptor and Muller glia cultures express IL-27 receptors, and IL-27 incubation led to STAT1-dependent induction of IL-10 and SOCS1. The authors concluded that IL-27 produced by retinal neurons and glia contributed to the neuroprotective effects of IL-27 in vivo.

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latter, IL-10 induces STAT3/AKT signaling, leading to increased transcription of the anti-apoptotic genes Bcl-2 and Bcl-XL and increased survival of cultured neurons exposed to the elevated glutamate (Zhou et al., 2009). IL-10 also leads to inactivation of GSKβ, raising the possibility that IL-27/IL-10 signaling could stimulate the neuroprotective Wnt pathway (Garcia et al., 2018). Furthermore, the anti-inflammatory effect of IL-27 is lost or reduced in animals lacking IL-10 (Fonseca et al., 2019), indicating the importance of IL-10 signaling to IL-27 function. However, these studies suggest that IL-27/IL-10 signaling is not yet fully understood.

Figure 1 | Potential mechanisms of neuronal protection from IL-27 acting on innate immune cells and neurons.
Macrophages produce IL-27 after stimulation of TLR, CD40, IFN, and other receptors, via NF-κB or STAT1. The secreted IL-27 binds to receptors on microglia, dendritic cells, and astrocytes, which activates STAT1/3 signaling pathways, induction of anti-inflammatory proteins, reduction of pro-inflammatory proteins, and inhibition of the NLRP3 inflammasome. CD39 expression in dendritic cells is required for inflammasome inhibition in dendritic cells. IL-10 released from microglia binds to IL-10 receptors on neurons and stimulates anti-inflammatory pathways (Bcl-2, Bcl-XL). IL-27 has direct effects on neurons by binding to IL-10 receptors and stimulating anti-expression of IL-10 and TGFβ.

Cytokine expression
IL-27 reduces the expression of many inflammatory cytokines, and the combined effects of suppressed pro-inflammatory cytokines and elevated anti-inflammatory IL-10 may tip the balance to promoting an immunosuppressive and neuroprotective tissue environment. A study by Mascarenho et al. using the EAE model showed that IL-27 reduced pro-inflammatory cytokines in the CNS, validating the potential anti-inflammatory properties of IL-27 in the CNS. IL-27 reduced expression of pro-inflammatory cytokines in the CNS, indicating the importance of IL-27 in regulating innate and adaptive immune response, the mechanism by which IL-27 induces cell death is not entirely understood. A recent study demonstrated that IL-27/STAT3 signaling in the EAE mouse model induced apoptosis of CD44+T cells, which occurs by inducing PD-L1 on monocyte-derived dendritic cells that bind to programmed cell death protein 1 on pathogenic T cells (Casella et al., 2020). Furthermore, using the streptozotocin-induced mouse model of Alzheimer’s disease, Salem et al. (2021) demonstrated that treatment with the phosphodiesterase 5 inhibitor tadalafil and anti-inflammatory naturally derived product bergapten resulted in reduced IL-27 and IL-26 levels that were associated with decreased neuroinflammation, reduced neuronal loss, and improved cognitive function. These findings raise the possibility that IL-27 promotes degeneration in this mouse model and is suppressed by neuroprotective treatments.

The role of IL-27 in neuroinflammation in the BBR mouse model of autism was demonstrated (Ahmad et al., 2020). IL-27 induced NF-κB signaling in this mouse model led to reduced inflammation, and was associated with lower levels of splenic CD44+IL-27+ cells and other pro-inflammatory molecules, and decreased IL-27 expression in the CNS (Ahmad et al., 2017). These results suggest that IL-27 may be involved in microglial and neuroinflammatory responses underlying adenosine A2a receptor-regulated behaviors.

IL-27 induces apoptosis as a component of its anti-tumor function. For example, IL-27 induced apoptosis of B cells from patients with chronic lymphocyte leukemia, although did not induce apoptosis in B cells from control subjects (Manouchchehri-Doulabi et al., 2020). Furthermore, IL-27 promoted apoptosis of several tumor cell lines, including enhancing sensitivity to the chemotherapy drug cisplatin in a lung cancer cell line (Jiang et al., 2021) and increasing sensitivity to poly(l)-induced cell death in a prostate tumor cell line (Kourko et al., 2019). Evidence for its anti-tumor effects in vivo was shown using mouse models of colon, lung, and breast tumors, in which IL-27 over-expression led to improved survival by depleting Treg cells through STAT3 downregulation, leading IL-27 expression (Zhu et al., 2018). How the IL-27/STAT1 and STAT3 signaling pathways lead to survival or death in different cell types remains to be elucidated.

IL-27 also mediates pro-inflammatory effects by promoting proliferation and survival of CD44+ T cells, differentiation, and effector function of Th1 and Th17 cells (Fan et al., 2018). IL-27 has been shown to induce IL-10 and TGFβ, reduced inflammatory molecules, and decreased IL-27 expression in the CNS (Ahmad et al., 2017). Although the majority of studies indicate anti-inflammatory and anti-apoptotic effects of IL-27 in the CNS, developing the IL-27 cytokine as a potential neuroprotective therapy may be complicated by its pro-inflammatory and neuro-death activities observed in certain cell types and disease models. Although some of these pro-death activities are beneficial and limit an overactive immune response, the mechanism by which IL-27 induces cell death is not entirely understood. A recent study demonstrated that IL-27/STAT3 signaling in the EAE mouse model induced apoptosis of CD44+ T cells, which occurs by inducing PD-L1 on monocyte-derived dendritic cells that bind to programmed cell death protein 1 on pathogenic T cells (Casella et al., 2020). Furthermore, using the streptozotocin-induced mouse model of Alzheimer’s disease, Salem et al. (2021) demonstrated that treatment with the phosphodiesterase 5 inhibitor tadalafil and anti-inflammatory naturally derived product bergapten resulted in reduced IL-27 and IL-26 levels that were associated with decreased neuroinflammation, reduced neuronal loss, and improved cognitive function. These findings raise the possibility that IL-27 promotes degeneration in this mouse model and is suppressed by neuroprotective treatments.

Pro-Inflammatory and Apoptotic Activities of Interleukin-27

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In contrast, IL-27 did not alter IL-10 or SOCS-3 levels in cultured microglia co-treated with oncostatin M (OSM) but reduced TNFa and iNOS and inhibited NF-κB activation (Baker et al., 2010). Lower levels of inflammatory cytokines by IL-27 treatment were associated with decreased OSM-induced death of cultured mouse primary cortical neurons (Baker et al., 2010). The mechanism by which IL-27 inhibits NF-κB in these cells is unclear but it may involve altered binding to the p65 subunit that IL-27 and OSM both use (Baker et al., 2010). Interestingly, IL-27 was shown to induce epigenetic changes in microglia by reducing binding of coactivator p300, decreased acetylation of histone H3 in TNFa and iNOS promoters, and increased binding of the transcriptional corepressor HDAC3 (Baker et al., 2010). Additionally, IL-27 induces several transcription factors, including c-Maf, Egr-2, IRF1, and BATF, which alter chromatin accessibility at promoter regions and induce transcription of target genes that mediate the effects of IL-27, such as IL-10 and genes involved in T cell function (Karwacz et al., 2017). Therefore, these studies suggest that IL-27 is a component of the tissue response that restores homeostatic levels of inflammation.

Conclusions and Future Directions

Precise control of neuroinflammatory signals is essential to restore tissue homeostasis after CNS injury — too little inflammation would reduce protective immune responses that remove dying neurons and limit damage, whereas too much inflammation impairs neuronal survival and worsens tissue damage. Research into the roles of IL-27 in regulating innate and adaptive immunity pathways that protect the CNS from neurodegeneration is still in its early stages. Several studies show that delivering functional IL-27 to the retina or brain reduces excessive neuroinflammation through several mechanisms and decreases neuronal death. IL-27 also directly stimulates STAT3-mediated survival pathways upon binding to IL-10Rα on neurons. It is important to note that while the majority of studies indicate anti-inflammatory and anti-apoptotic effects of IL-27 in the CNS, the pro-inflammatory function associated in certain disease models must be properly understood before developing IL-27 as a potential therapy. Key questions to be investigated are: (1) how IL-27...
expression and activity are regulated after acute and chronic injury. (2) what is the role of IL-27 in tissues with "immune privilege", such as the retina, (3) how IL-27 secretion recruits the activity of other immune cells within the CNS in the immediate stages after injury, (4) identification of the signaling pathways that regulate the protective effects of IL-27, (5) what is the contribution of IL-27 signaling within other CNS cell types, such as radial glia, astrocytes, and neurons, to the overall tissue response, and (6) what downstream signals determine whether IL-27 acts as anti-apoptotic, as seen in neuronal tissue, or pro-apoptotic, as seen in tumor cells. In conclusion, further investigation of the promising cytoprotective and immunomodulatory properties of the IL-27 cytokine is warranted and may ultimately lead to the development of novel therapeutic strategies for diseases of the CNS and retina.

Search Strategy and Selection Criteria

The following search terms were used in PubMed for preparing this narrative review: "IL-27", "EB3i", "WSX-1", "IL-10" and "IL-27r" in combination with "CNS", "neuron", "brain", "retina", "spinal cord", "neurodegenerative disease", "neuroinflammatory disease", "survival", "or specific diseases ("Alzheimer's disease", "Parkinson's disease", "multiple sclerosis", etc.), or in combination with specific cell types ("microglia", "macrophage", "dendritic cell", "neuron", "neutrophil", "astrocyte", "T cell") or in combination with specific mechanisms ("autobiological", "mechanism", "all year apopoptosis") were chosen in the search. These searches were performed between July and September 2021.

Author contributions:
The manuscript was conceived and designed by ASH. Literature review, manuscript writing and editing were performed by ASH, ANW, and KMA. All authors read and approved the final manuscript.

Conflicts of Interest:
There are no conflicts of interest.

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