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Cytokines and Chemokines in Neuromyelitis Optica: Pathogenetic and Therapeutic Implications

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Abstract

Neuromyelitis optica (NMO) is characterized by severe optic neuritis and longitudinally extensive transverse myelitis. The discovery of an NMO-specific autoantibody to the aquaporin-4 (AQP4) water channel has improved knowledge of NMO pathogenesis. Many studies have focused on inflammatory and pathological biomarkers of NMO, including cytokines and chemokines. Increased concentrations of T helper (Th)17- and Th2-related cytokines and chemokines may be essential factors for developing NMO inflammatory lesions. For example, interleukin-6 could play important roles in NMO pathogenesis, as it is involved in the survival of plasmablasts that produce anti-AQP4 antibody in peripheral circulation and in the enhancement of inflammation in the central nervous system. Therefore, assessment of these useful biomarkers may become a supportive criterion for diagnosing NMO. Significant advances in the understanding of NMO pathogenesis will lead to the development of novel treatment strategies. This review focuses on the current advances in NMO immunological research, particularly that of cytokines and chemokines.

INTRODUCTION

Neuromyelitis optica (NMO) is an autoimmune inflammatory disorder of the central nervous system (CNS), clinically presenting with longitudinally extensive transverse myelitis (LETM) and optic neuritis (54). The discovery of the disease-specific serum anti-aquaporin-4 (AQP4) antibody in NMO (25, 26) has dramatically changed the clinical definition of NMO, leading to recent advances in NMO research. The pathogenic role of anti-AQP4 antibody was demonstrated *in vivo* by passive transfer experiments in animal models (7, 22, 34). Several lines of evidence differentiating between NMO and multiple sclerosis (MS) have accumulated based on pathology (30, 33), neuroimaging (16), immunological findings (38) and responses to immunotherapies (24, 29, 39). On the basis of these extensive data, NMO is now considered an anti-AQP4 antibody-mediated astrocytopathy distinct from demyelinating disorders as represented by MS (12). Besides anti-AQP4 antibody, many additional biomarkers have proven useful for understanding the pathogenetic and immunological aspects of NMO (32, 36, 38). T and B cells may be implicated in the peripheral/CNS immune responses and pathogenesis of NMO, whereas various cytokines and chemokines have also been associated with the pathogenesis of NMO (38). Therefore, this review focuses on the current research on the roles of cytokines and chemokines in NMO pathogenesis and their therapeutic applications.

Cerebrospinal fluid (CSF) cytokines and chemokines in NMO patients

Many studies have analyzed CSF cytokine and chemokine levels in NMO patients (Table 1). Although some cytokines may increase nonspecifically because of CNS inflammation, several cytokines and chemokines are directly related to NMO pathogenesis. T helper (Th)17- and Th2-related cytokines are upregulated in the CSF of NMO patients (38). CSF interleukin (IL)-17 levels increase in patients with NMO (48) or opticospinal MS (OSMS; some of whom were considered to have NMO) (15, 37). Many studies have also shown increased CSF IL-6 levels in patients with NMO. Presumably, NMO expresses Th17 and Th2 axes in CNS (Figure 1) differently from MS, which is primarily a Th1-dominant disease. However, further studies are necessary to clarify the definite cytokine and chemokine profiles in NMO.

Th17-related cytokines and chemokines

IL-17 is involved in the development of autoimmune diseases and acts as a potent mediator in delayed-type inflammatory reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the inflammation site. As described earlier, several reports have revealed elevated CSF IL-17 levels in NMO (48) or OSMS patients (15, 37). Our own study could not confirm such an elevation, but levels of some Th17-related cytokines and chemokines are reportedly increased in NMO patients (38).

Elevated CSF IL-6 levels in NMO have also been reported. IL-6 is a proinflammatory cytokine with a wide variety of functions. Secreted by immunocytes and activated astrocytes (11), it promotes immunoglobulin (Ig) synthesis in activated B cells and differentiation of naïve T cells into Th17 cells or cytotoxic T cells (6, 23). Among the several CSF cytokines and chemokines elevated in NMO, IL-6 shows the strongest correlation with clinical variables in NMO; these include CSF glial fibrillary acidic

Table 1. Cerebrospinal fluid (CSF) cytokine/chemokine levels in NMO patients.

CSF cytokines/chemokines	Axis	Change	Reference	Correlation
IL-17	Th17	↑ (vs. MS, ONNDs)	(48)	CSF HMGB1
IL-6	Th17	↑ (vs. MS, HC)	(9)	
		↑ (vs. MS, ONNDs)	(14)	EDSS and AQP4 ab positivity
		↑ (vs. MS)	(55)	Definite form > limited form
		↑ (vs. MS, ONNDs)	(41)	CSF cells, CSF proteins and AQP4 ab positivity
		↑ (vs. MS, ONNDs)	(38) (44)	CSF cells, CSF GFAP, AQP4 ab, recovery from relapse and relapse duration
		↑ (vs. MS, ONNDs)	(43)	CSF HMGB1
		↑ (vs. MS, ONNDs)	(49)	EDSS
		↑ (vs. MS, ONNDs)	(48)	CSF HMGB1
IL-1ra	Th17	↑ (vs. MS, ONNDs)	(38)	CSF cells and CSF GFAP
G-CSF	Th17	↑ (vs. MS, ONNDs)	(38)	CSF cells and CSF GFAP
IL-8	Th17	↑ (vs. MS, ONNDs)	(38)	CSF cells, CSF GFAP and EDSS
		→ (vs. MS)	(55)	
IL-13	Th2	↑ (vs. MS, ONNDs)	(38)	CSF cells and CSF GFAP
IL-5	Th2	↑ (vs. MS, HC)	(9)	
Eotaxin-2, -3	Th2	↑ (vs. MS, HC)	(9)	
Eotaxin	Th2	→ (vs. MS, ONNDs)	(38)	
		→ (vs. MS, ONNDs)	(31)	
TARC	Th2	↑ (vs. ONNDs)	(31)	
IL-10	Treg	↑ (vs. ONNDs) → (vs. MS)	(38)	CSF cells, AQP4 ab and CSF GFAP
		→ (vs. MS)	(55)	
IL-12	Th1	↑ (vs. HC)	(9)	
		→ (vs. MS, ONNDs)	(38)	
IL-1β	Th1	↑ (vs. MS)	(55)	Definite form > limited form
		→ (vs. MS, ONNDs)	(38)	
CXCL10 (IP-10)	Th1	↑ (vs. ONNDs)	(31)	
		↑ (vs. ONNDs)	(38)	CSF cells and CSF GFAP
CXCL13	B cell	↑ (vs. MS, ONNDs)	(57)	ARR and EDSS
IFN-γ, G-CSF, IL-17		↑ (vs. CMS, ONNDs)	(37)*	
IL-17, MIP-1β, IL-1β, IL-13, IL-8, IL-10, TNF-α, IL-5		↑ (vs. ONNDs)	(15)*	IL-8: EDSS, albumin quotient and length of spinal cord lesion
		↑ (vs. CMS)		IL-17: albumin quotient and length of spinal cord lesion

*Cytokine analyses were performed in opticospinal MS patients.

AQP4 ab = aquaporin-4 antibody; ARR = annualized relapse rate; CMS = conventional multiple sclerosis; CXCL = (C-X-C motif) ligand; EDSS = Expanded Disability Status Scale; G-CSF = granulocyte colony-stimulating factor; GFAP = glial fibrillary acidic protein; HC = healthy controls; HMGB1 = high mobility group box 1; IFN-γ = interferon-gamma; IL = interleukin; IP-10 = interferon gamma-induced protein 10; MIP = macrophage inflammatory protein; MS = multiple sclerosis; NMO = neuromyelitis optica; ONNDs = other noninflammatory neurological disorders; TARC = thymus and activation-regulated chemokine; Th = T helper; TNF-α = tumor necrosis factor-alpha; Treg = regulatory T cell.

↑ = upregulation; → = unchanged.

protein (GFAP) levels, CSF cell counts and anti-AQP4 antibody titers (38). Içöz *et al* reported that patients with NMO have higher CSF IL-6 levels than those with optic neuritis, relapsing–remitting MS or healthy control (HC). Further, CSF IL-6 levels in NMO patients correlate with anti-AQP4 antibody titers and the Expanded Disability Status Scale (EDSS) score (14). Wang *et al* found that CSF IL-6 and soluble IL-6 receptor levels are significantly higher in patients with NMO than in those with MS and other noninflammatory neurological disorders (ONNDs) (49). Yanagawa *et al* reported elevated CSF IL-6 levels in patients with definite NMO compared with those with limited NMO (anti-AQP4-positive myelitis without optic neuritis) (55). The CSF/serum ratio of IL-6 is significantly higher in NMO than in ONNDs, suggesting that IL-6 is mainly produced in the CNS of NMO patients (38). Although IL–6-producing cells in CNS have not yet been identified, activated or damaged, astrocytes by anti-AQP4 antibody may produce IL-6 in the CNS of NMO patients. Of note, high CSF IL-6 levels have been found in 82.3% of NMO patients, but no such increase has been observed in MS patients (38). CSF IL-6 levels are also markedly high not only during relapse, but also during the initial attacks in NMO patients (45). Interestingly, CSF IL-6 levels can predict recovery from NMO relapses and relapse-free duration (44). NMO patients who relapse with optic neuritis exhibit high CSF IL-6 levels, similar to NMO patients who relapse with myelitis (38, 45); nevertheless, optic neuritis lesions are usually much smaller than myelitis lesions in NMO patients. These data suggest that CSF IL-6 is not a product of NMO inflammation, but an important molecule in the pathology of this disease. We have recently shown that CSF IL-6 levels correlate with CSF levels of high mobility group box 1 (HMGB1), a proinflammatory mediator (43), and with CSF-soluble intercellular adhesion molecule 1 levels, one of the markers of blood–brain barrier disruption

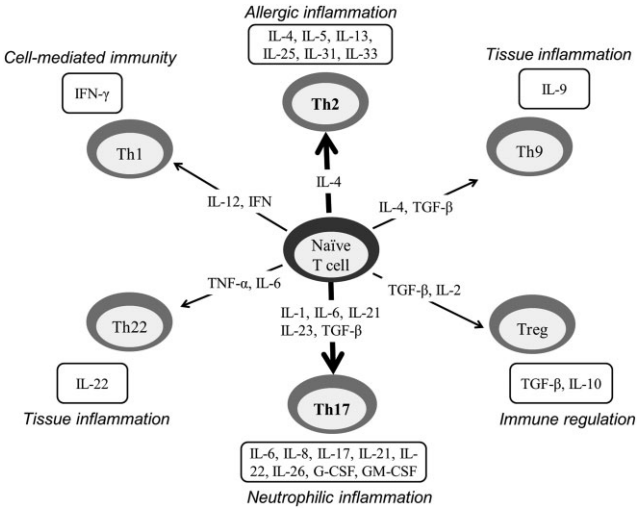

Figure 1. *Differentiation pathways of naïve CD4⁺ T cells.* CD4⁺ T cells can differentiate into T helper (Th)1, Th2, Th9, Treg, Th17 or Th22 by the actions of differentiation cytokines. These T-cell subsets promote different inflammatory responses based on their respective cytokine profiles, responses to chemokines and interactions with other cells. The Th17 and Th2 axes may be mainly upregulated in neuromyelitis optica. IL = interleukin; TGF = transformaing growth factor; TNF = tumor necrosis factor.

(42). Accumulated evidence suggests important roles for CSF IL-6 in NMO pathogenesis; these could include CNS inflammation, astrocytic damage and blood–brain barrier disruption. In addition, CSF IL-6 may serve as a biomarker to diagnose NMO and differentiate it from MS. It remains unclear whether astrocytic damage releases IL-6, or IL-6 directly contributes to astrocytic damage and CNS inflammation in NMO.

IL-1ra is a member of the IL-1 cytokine family. CSF IL-1ra levels are significantly elevated in NMO compared with MS or ONNDs and correlate with CSF cells and CSF GFAP levels (38). Granulocyte colony-stimulating factor (G-CSF) stimulates survival, proliferation and differentiation of neutrophils. CSF G-CSF levels are higher in NMO than in MS and ONNDs and correlate with CSF GFAP levels and CSF cell counts (38). Tanaka *et al* reported significantly elevated CSF G-CSF levels in patients with OSMS (some of whom were considered to have NMO) compared with conventional MS (CMS) and ONNDs, and found correlations with the albumin quotient, length of spinal magnetic resonance image (MRI) lesions and EDSS score (37). IL-8 is known as a neutrophil chemotactic factor. CSF IL-8 levels are significantly elevated in NMO compared with MS and ONNDs, and correlate with the CSF GFAP levels, CSF cell counts and EDSS score (38). However, Yanagawa *et al* reported no difference between CSF IL-8 levels in NMO and MS patients (55). Ishizu *et al* found that significantly elevated CSF IL-8 levels in OSMS patients (some of whom were considered to have NMO) compared with CMS and ONNDs. These levels correlate with the albumin quotient, length of spinal MRI lesion and EDSS score (15). They speculated that the markedly increased IL-8 in CSF may be relevant to neutrophil infiltration in CNS.

Th2-related cytokines and chemokines

Although IL-4, a representative Th2-related cytokine, has not been elevated in CSF (38), other Th2-related cytokines and chemokines are upregulated in the CSF of NMO patients. The effects of IL-13 on immune cells are similar to those of IL-4. CSF IL-13 levels are elevated in NMO compared with MS or ONNDs, and their levels correlate with CSF cells and CSF GFAP levels (38). IL-5 stimulates B-cell growth and increases Ig secretion; it is also a key mediator in eosinophil activation. CSF IL-5 levels are significantly higher in NMO patients than in MS patients or HC (9). Eotaxin is an eosinophil-selective chemokine. CSF eotaxin levels in NMO patients are similar to those in MS or ONNDs patients (31, 38). However, Correale and Fiol reported significant increases in CSF eotaxin-2 and eotaxin-3 levels in NMO patients compared with MS patients or HC (9). The chemokine thymus and activation-regulated chemokine (TARC) specifically binds and induces chemotaxis in T cells. CSF TARC levels are significantly higher in NMO than in ONNDs (31).

Th1-related cytokines and chemokines

Interferon-gamma (IFN-γ), a representative Th1-related cytokine, has not been elevated in the CSF of NMO patients (38). (C-X-C motif) ligand (CXCL10) (interferon gamma-induced protein 10) is secreted by several cells in response to IFN-γ. CSF CXCL10 levels are significantly higher in NMO than in ONNDs (31, 38), and correlate with CSF GFAP levels and CSF cell counts (38). IL-12 is involved in the differentiation of naïve T cells into Th1 cells and plays an important role in the activities of natural killer cells and T lymphocytes. CSF IL-12 levels are significantly elevated in NMO patients compared with HC (9). However, some studies report no differences in CSF IL-12 levels between NMO, MS or ONNDs (38). IL-1β is an important mediator of the inflammatory response. CSF IL-1β levels are elevated in patients with definite NMO compared with those with limited NMO (55). However, no differences have been found between NMO, MS or ONNDs patients (38).

Other cytokines and chemokines

IL-10, a regulatory T (Treg)-related cytokine with pleiotropic effects in immunoregulation and inflammation, is capable of inhibiting proinflammatory cytokine synthesis. CSF IL-10 levels are significantly elevated in NMO compared with ONNDs (38), but no difference is observed in MS patients (38, 55). CSF IL-10 levels correlate with CSF GFAP levels, CSF cell counts and anti-AQP4 antibody titers (38).

CXCL13 is selectively chemotactic for B cells. CSF CXCL13 levels are significantly higher in NMO than in MS or ONNDs and correlate with the annualized relapse rate and EDSS score (57). Alvarez *et al* reported elevated CSF CXCL13 levels in NMO and MS patients compared with ONNDs, which correlate with CSF cell counts in NMO patients (1).

Serum/plasma cytokines and chemokines in NMO patients

Serum/plasma cytokine and chemokine levels in NMO patients are summarized in Table 2. As with CSF analyses, Th17- and

Table 2. Serum cytokine/chemokine levels in NMO patients.

Serum cytokines/ chemokines	Axis	Change	Reference	Correlation
IL-17	Th17	↑ (vs. HC)	(51)	
		↑ (vs. HC)	(52)	HMGB1
		↑ (vs. HC)	(27)	
		↑ (vs. MS, HC)	(50)	
		↑ (vs. NMO without LSCL)	(13)	Length of spinal cord lesion
IL-6	Th17	↑ (vs. HC)	(28)	EDSS
		↑ (vs. MS, HC)	(9)	
		↑ (vs. MS, ONNDs)	(14)	
		↑ (vs. ONNDs)	(38)	
		↑ (vs. HC)	(47)	IL-32
		↑ (vs. HC)	(46)	
		↑ (vs. HC)	(51)	
IL-23	Th17	↑ (vs. HC)	(27)	
		↑ (vs. HC)	(50)	
		↑ (vs. HC)	(28)	
IL-21	Th17	↑ (vs. HC)	(50)	
		↑ (vs. HC)	(28)	EDSS
IL-4	Th2	↑ (vs. HC)	(2)	
		↑ (vs. MS, HC)	(51)	
IL-10	Treg	↑ (vs. MS, HC)	(51)	
IL-2	Treg	↑ (vs. MS, HC)	(51)	
		↓ (vs. HC)	(28)	
IFN-γ	Th1	↑ (vs. HC)	(52)	HMGB1
		↑ (vs. MS, HC)	(51)	
		↓ (vs. HC)	(28)	
TNF-α	Th1	↑ (vs. HC)	(52)	HMGB1
		↑ (vs. HC)	(51)	
IL-32		↑ (vs. MS, HC)	(47)	IL-6, EDSS

EDSS = Expanded Disability Status Scale; HC = healthy controls; HMGB1 = high mobility group box 1; IFN-γ = interferon-gamma; IL = interleukin; LSCL = long spinal cord lesions greater than three vertebral segments; MS = multiple sclerosis; NMO = neuromyelitis optica; ONNDs = other noninflammatory neurological disorders; Th = T helper; TNF-α = tumor necrosis factor-alpha; Treg = regulatory T cell.
↑ = upregulation; ↓ = downregulation.

Th2-related cytokines and chemokines are predominantly upregulated in the serum/plasma of NMO patients (Figure 1).

Serum/plasma IL-17 levels increase in NMO patients compared with HC or MS patients (27, 50–52). Plasma IL-17 levels correlate with plasma HMGB1 levels (52). NMO patients with LETM (more than three vertebral segments) have higher serum IL-17 levels than NMO patients without LETM (13). NMO patients in the relapse phase have significantly higher serum IL-6 levels than ONND patients (38). Içöz *et al* also reported that patients with NMO, particularly those who are anti-AQP4 antibody positive, have higher serum IL-6 levels than those with optic neuritis, relapsing–remitting MS or HC (14). Wang *et al* found that plasma IL-6 levels are higher in NMO patients than in HC and are positively correlated with IL-32 levels (47). AQP4-specific T-cell responses are amplified in NMO patients and exhibit a Th17 bias, and intracellular IL-6 production increases after lipopolysaccharide stimulation in monocytes from NMO patients (46). The number of anti-myelin oligodendrocyte glycoprotein IL-6- and IL-12-secreting cells in the peripheral blood and CSF of NMO patients is higher than that of MS, ONNDs or HC (9). The release of IL-6, IL-21 and IL-23 from activated peripheral blood mononuclear cells is significantly higher in NMO patients than in controls, and IL-6 and IL-21 levels positively correlate with the EDSS score in NMO patients (28). Although the role of IL-6 in peripheral blood is unclear, Chihara *et al* recently reported that the population of plasmablasts exhibiting the $CD19^{int}CD27^{high}CD38^{high}CD180^{-}$ phenotype selectively increases in the peripheral blood of NMO patients, and that these plasmablasts are major producers of anti-AQP4 antibodies (8). IL-6 enhances plasmablast survival and anti-AQP4 antibody production in these cells, whereas anti-IL-6 receptor antibody lessens their survival. IL-6 in the peripheral blood of NMO patients is implicated in the peripheral immune response and anti-AQP4 antibody production. Serum IL-23 and IL-21 levels are also elevated in NMO patients compared with HC (27, 50).

The Th2 cytokine IL-4 is upregulated in the serum of NMO patients compared with HC and MS patients (2, 51). Other Th2-related cytokines and chemokines have not been analyzed.

Studies of Treg-related cytokines show that IL-10 and IL-2 levels increase significantly in NMO patients compared with MS patients and HC (51), but Linhares *et al* reported that IL-2 levels decrease significantly in NMO patients compared with controls (28).

The levels of the Th1-related cytokines IFN-γ and tumor necrosis factor-alpha (TNF-α) increase in NMO patients compared with HC and MS patients (51, 52), and are correlated with plasma HMGB1 levels (52).

Chemokine receptor expression on peripheral blood T cells in NMO patients

CD8+CXCR3+T cells might affect the pathogenesis of both NMO and MS, and could be an important marker of disease activity. The CD8+CXCR3+/CD8+CCR4+ ratio, which reflects immune and inflammatory activities, is higher in NMO than in MS patients (35). Th1 dominance of chemokine receptors on blood T cells and the correlation between CXCR3+ T cells and disease activity have been confirmed by analyzing chemokine receptors on peripheral blood lymphocytes during the relapse phase in MS patients. However, such deviations in the Th1/Th2 balance have not been observed in NMO patients (40).

Pathogenic role of cytokines and chemokines

IL-6 infusion into the spinal subarachnoid space of rats induces progressive weakness with CNS inflammation, axonal degeneration and myelin loss (18). CSF IL-6 is mainly produced by astrocytes in transverse myelitis patients, and its levels correlate with astrocytic expression and disease severity (18). IFN-β treatment is effective in reducing experimental autoimmune encephalomyelitis (EAE) symptoms induced by Th1 cells, but exacerbates disease induced by Th17 cells (4). The Th17 EAE model represents several aspects of NMO, suggesting that Th17 cells may play a pathogenic role in NMO pathogenesis. *Ex vivo* experiments performed on murine spinal cords have revealed that slices exposed to NMO IgG and human complement exhibit NMO-like lesions. These lesions increase in severity with the addition of neutrophils, natural killer cells, macrophages or cytokines (such as TNF-α, IL-6, IL-1β or IFN-γ), implicating specific immune cells and cytokines may amplify tissue damage in NMO (56).

Therapeutic implications of cytokine blockade in NMO patients

Low-dose oral corticosteroids, azathioprine, mitoxantrone, cyclophosphamide, mycophenolate mofetil and rituximab are used as maintenance treatments to prevent NMO relapses (10, 17, 20, 21, 53). Novel treatments using the IL-6 pathway blocker tocilizumab, a recombinant humanized monoclonal antibody against the IL-6 receptor, may be useful for suppressing relapses in NMO patients who cannot tolerate standard immunosuppression therapy (3, 5, 19). Treatment with tocilizumab rapidly reduces the number of elevated plasmablasts and anti-AQP4 antibody titers in NMO patients. Furthermore, neuropathic pain and disability scores improve gradually (3). Patients with highly active anti-AQP4 antibody-positive NMO, in whom numerous immunosuppressive interventions had failed, exhibited improved EDSS scores and annualized relapse rates after initiating tocilizumab. Tocilizumab significantly reduces CSF IL-6 levels, signal transducer and activation of transcription 3 (STAT3) activation (19). Three female patients with anti-AQP4 antibody-positive NMO, who were resistant to rituximab treatment, exhibited a decrease in the median annualized relapse rate from 3.0 to 0.6 after treatment with tocilizumab (5). IL-6 receptor-blocking therapy can be effective against NMO even in patients who fail to respond to conventional therapy. This direct clinical evidence suggests that IL-6 may be a critical molecule in NMO immunopathogenesis. In the future, other cytokine-blocking therapies may also be applied clinically.

CONCLUSIONS

A growing number of recent immunological studies have supported the important role of cytokines and chemokines in NMO pathogenesis. Although many cytokines and chemokines are upregulated in both the peripheral and CNS of NMO patients, Th17- and Th2-related cytokines and chemokines, particularly Th17-related cytokines, may be key players in NMO inflammation. IL-6 in the peripheral blood is implicated in anti-AQP4 antibody production in NMO patients, and IL-6 in CSF plays important roles in CNS inflammation, astrocytic damage and blood–brain barrier disruption. Thus, IL-6-blocking therapy with tocilizumab may be a promising treatment option for NMO patients. New treatments need to be developed to prevent severe relapses in these patients. A better understanding of the role of cytokines and chemokines in NMO pathogenesis is critical to developing effective treatments.

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