Angiotensin II Receptor Type 1 Expression in Erythroid Progenitors: Implications for the Pathogenesis of Postrenal Transplant Erythrocytosis

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Under normal physiological conditions red blood cell production is controlled primarily by erythropoietin, although multiple additional stimulatory factors are likely to be involved. One of these factors, angiotensin II, can modulate erythropoiesis directly via its type 1 receptor, as well as indirectly through multiple secondary mediators. We propose that angiotensin II exerts its stimulatory effect during the early stages of erythropoiesis, and that this effect serves as an important compensatory mechanism if erythropoietin production is chronically inadequate. We speculate that if this compensatory stimulation continues to be abnormally high after restoration of erythropoietin production following renal transplantation, erythrocytosis ensues.

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ERYTHROPOIESIS IS a dynamic process ensuring adequate oxygen delivery to tissues by maintaining a sufficient number of circulating red blood cells. From only a few undifferentiated erythroid progenitors arising from a pluripotent hematopoietic stem cell, a large number of erythroblasts can be generated in multiple steps closely regulated by cytokines and hormones, assuring the necessary number of red blood cells for optimal oxygen delivery to the tissues. In normal individuals, the rate of erythropoiesis can increase substantially during various physiological challenges such as acute blood loss or hypoxia. When the controlling mechanisms are dysregulated (e.g., polycythemia vera, or mutations of the erythropoietin (Epo) receptor (EpoR) or von Hippel Lindau (VHL) genes), an increased number of red blood cells (polycythemia/erythrocytosis) or a decreased number of red cells (anemia) ensues.

MODULATORS OF ERYTHROPOIESIS

Erythropoietin (Epo) is an essential factor for regulation of proliferation, differentiation, and prevention of apoptosis of erythroid cells.1-3 Hypoxia is the principal regulator of Epo production. Hypoxia inducible factor (HIF) is a transcription factor that is the master regulator of Epo production, as well as a multitude of hypoxia-controlled genes. In hypoxia or as a result of a mutated VHL gene,4 the accumulation of the undegraded HIF-1α leads to the formation of a heterodimer with HIF-1β and the activation of an array of hypoxia-inducible genes (including EPO) and enhanced erythropoiesis takes place.5 The VHL protein (pVHL) plays a crucial role in hypoxia sensing.5 pVHL binds to the hydroxylated form of the HIF-1α and serves as the recognition component of an E3-ubiquitin ligase complex that comprises elongins B and C, cullin 2, and ring-box 1,6-8 thus assuring the downregulation of EPO by normoxia and the upregulation of its transcription by hypoxia.

However, it is clear other factors play an important role in the regulation of erythropoiesis. Although Epo has the dominant stimulatory influence on proliferation of the more mature erythroid progenitors, proliferation of the early progenitors is stimulated by complex interactions of various factors not unique to erythropoiesis (eg, insulin-like growth factor 1 [IGF-1], granulocyte-macrophage colony stimulating factor (GM-CSF), thrombopoietin, IL-6, IL-1, activin-A, basic fibroblast growth factor, androgens, angiotensin II [AngII], IL-3, and stem cell factor [c-kit])9-15 (Fig 1). In contrast, N-acetyl-seryl-aspartyl-lysin-proline (Ac-SDKP), a naturally occurring, circulating tetrapeptide, can significantly inhibit erythropoiesis by suppressing proliferation of stem cells,16 as well as by release of cytokines such as TGF-β.17

THE RENIN–ANGIOTENSIN SYSTEM IN ERYTHROPOIESIS

The role of the renin–angiotensin system (RAS) in the regulation of erythropoiesis has been long suspected, although the controlling mechanisms are complex and not fully elucidated. The RAS was first postulated to modulate erythropoiesis in 1980s after discovery of an association of anemia...
with the use of angiotensin-converting enzyme (ACE) inhibitors (ACE-I) for treatment of hypertension.\textsuperscript{18-20} The ACE-I–related anemia was most pronounced in patients with renal insufficiency or end-stage renal disease (ESRD)\textsuperscript{18,21} and in patients with a renal allograft.\textsuperscript{22-26} The pathogenesis of this anemia was not clear, but reduced levels of circulating Epo did not appear to be solely responsible,\textsuperscript{26-32} suggesting that other factors must contribute.

Subsequently, AngII was shown to significantly modulate erythropoiesis. Although AngII directly stimulated proliferation of hematopoietic progenitors in vitro (eg, BFU-E),\textsuperscript{13,33} inhibition of its effects with ACE-I–induced apoptosis of erythroid progenitors in renal transplant patients.\textsuperscript{34} In an in vivo laboratory model, mice with ACE gene knockout developed normocytic anemia that was fully reversed with AngII infusion.\textsuperscript{35} These mice had elevated circulating levels of Epo and AcSDKP.

Does Angiotensin II Stimulate Secretion of Erythropoietin?

The search for a mechanism for AngII to increase erythropoiesis initially focused on a possible effect on Epo synthesis. In normal animals, increased blood levels of renin (a major regulator of AngII synthesis), induced by infusion or other means, increased serum Epo levels.\textsuperscript{36-40} In humans, infusion of AngII increased serum Epo levels.\textsuperscript{41,42} This effect was reversed by losartan, an AngII receptor type 1 (AT1R) blocker (ARB), suggesting that the AngII effect on Epo levels was modulated by the AT1R. The pathway underlying the AngII-driven Epo secretion is unknown. However, some investigators have suggested that AngII modulates renal Epo production through changes in renal perfusion and sodium reabsorption.\textsuperscript{43} This hypothesis is based on the presumption that reduced oxygen pressure in the kidneys triggers HIF-1α to induce release of Epo.\textsuperscript{44}

Angiotensin II Effect on Erythroid Progenitors

AngII can increase erythropoiesis also by an Epo-independent stimulation of erythroid progenitors. AngII has the propensity to act as a mitogen in various tissues, including smooth muscle,\textsuperscript{43,45,46} epidermal stem cells,\textsuperscript{47} and hematopoietic progenitors. Moreover, it can promote mitogenesis and growth by release of various growth factors, including TGF-β\textsuperscript{48} and PDGF-A.\textsuperscript{49} A direct stimulatory effect on erythropoiesis was demonstrated in vitro with normal early erythroid progenitors derived from CD34+ cells isolated from peripheral blood,\textsuperscript{33} bone marrow, and cord blood.\textsuperscript{33} This stimulatory effect was reversed by an ARB, losartan. In lineage uncommitted progenitors (Lin−) in murine bone marrow, AngII increased the proliferation of hematopoietic cell progenitors in the presence or absence of colony-stimulating factors. This effect was also reversible with losartan.\textsuperscript{33} Thus, AngII can alter proliferation of hematopoietic progenitors directly by binding to AT1R on progenitor cells as well as indirectly by inducing an AT1R-modulated release of hematopoietic growth factors from non-erythroid cells (eg, bone marrow stromal cells).

In contrast to Epo’s effect on the more mature erythroid progenitors (ie, colony-forming unit--
erythroid; CFU-E), the stimulatory effect of AngII on erythropoiesis is primarily directed toward the early erythroid progenitors (ie, burst-forming unit–erythroid; BFU-E). This effect appears to be mediated through AT1R that are expressed on these immature cells.

The magnitude of the contribution of AngII to erythropoiesis is not known in most individuals. However, in clinical settings of “functional Epo deficiency” (eg, renal failure), the Epo-augmenting impact of the AngII effect could be significant. In many renal transplant patients, erythropoiesis could be AngII-dependent and if treatment of hypertension or proteinuria with ACE-I is undertaken, the inhibitory effect on erythropoiesis might be particularly pronounced. This therapy induces Fas-, FADD-, and TADD-mediated apoptosis of CD34+ erythroid progenitor cells. Although ACE-I therapy can decrease hematocrits in renal transplant patients and normal control subjects, apoptosis was not observed in the latter. In patients with cancer, a prominent role of AngII in early erythropoiesis is also suggested by its ability to accelerate the recovery of hematopoietic progenitors after chemotherapy and radiation.

In addition to increasing the supply of AngII, ACE likely augments erythropoiesis by a second mechanism. This enzyme inactivates Ac-SDKP, a naturally occurring peptide that prevents recruitment of hematopoietic stem cells and early progenitors into the S-phase of the start of the synthesis of red blood cells.

Possible Biochemical Pathways in AT1-Mediated Erythropoiesis

AngII receptors type 1 (AT1R) and type 2 (AT2R) are coupled with G-proteins; however, their activation results in complex functional interactions. An opposite crosstalk of AT1R (synergistic) and AT2R (reductive) with epidermal growth factor (EGF) receptors on murine N1H3T3 fibroblasts demonstrated a role for AngII in activation of the mitogen-activated protein kinase (MAPK) pathway. Stimulation of this pathway is necessary for Epo-modulated proliferation of progenitor cells, and its activation can be achieved by the binding of the appropriate ligands to AT1R and EpoR. Moreover, AngII also activates the Jak2 kinase pathway. Although this effect was observed in smooth muscle cells, if a similar process occurs in hematopoietic cells, AngII would augment erythropoiesis by enhancing a Jak-2 kinase-mediated effect triggered by many erythroid growth factors such as Epo, IGF-1, GM-CSF, and IL-6. While stimulation of AT1R and EpoR leads to activation of additional “shared” regulatory pathways in erythroid cells, the one of functional relevance is the dose-dependent increase in cytosolic calcium levels (Fig 2). The importance of this common intracellular pathway stems from its potential role in mediating the Epo-augmenting effect of AngII.
from the fact that an isolated increase of calcium concentration in the culture media with constant levels of obligatory Epo increases the growth of BFU-E-derived cells by three-fold.66

Proposed Model of Epo- and AngII-Modulated Erythropoiesis

Although Epo has an overall critical role in the commitment and proliferation of erythroid progenitors,1 several other erythroid factors exert critical regulatory effects on erythropoiesis. AngII clearly facilitates Epo-driven proliferation pathways (eg, Jak-2 kinase, Ras, Raf, and increased cytosolic calcium levels); however, it can also stimulate erythropoiesis by increasing Epo production at either the systemic or local level. The ability of erythroid progenitors to overexpress AT1R suggests a potential to regulate their own proliferation, at least partly independent of systemic AngII and Epo levels. Although endocrine Epo production regulates the final number of generated red blood cells, autocrine Epo production67 can provide the critical amount of obligatory Epo necessary for erythroid progenitors during the later stages of their maturation.

POSTTRANSPLANT ERYTHROCYTOSIS

Polycythemias (erythrocytosis is a term also used, but no consensus has ever been reached about proper terminology) are characterized by an increased volume of red cell mass (erythron), and these can be either acquired or congenital, or, based on their pathophysiology, either primary or secondary.68 One of the acquired and secondary polycytemic disorders is posttransplant erythrocytosis (PTE). This syndrome is often defined as a persistent elevation of hematocrit (>51%) and is unique to recipients of renal allografts; its prevalence ranges from approximately 5-10%.30,69,70,77 PTE usually develops within 8 to 24 months after successful transplantation and resolves spontaneously within 2 years in approximately 25% of patients despite persistently good clearance function of the allograft.71 PTE can recur in the same patient after successful repeat transplantation.31 Other factors associated with development of PTE include male sex, lack of Epo therapy before transplantation, a history of smoking, diabetes mellitus, renal artery stenosis, lower serum ferritin levels,30 and normal or higher pretransplant Epo levels.30,72,73 PTE is also more frequent in patients who have been free of rejection,74 but is not associated with the ethnicity of the recipient or source of the allograft.75 At more extreme hematocrits (usually >60%), thrombotic events could complicate the clinical course.71

Although the molecular basis of PTE remains unknown, two major mechanisms of dysregulation of erythropoiesis have been postulated. The first mechanism assumes that PTE is mediated by increased availability27,69,70,76,77 or enhanced sensitivity of the erythroid progenitors26,30,31,50,71,78-80 to Epo. The second mechanism proposes that the RAS pathway increases Epo levels41,42 or directly stimulates proliferation of erythroid progenitors.13,47,81 Other factors could also contribute to the genesis of PTE. Androgens augment erythropoiesis through increased Epo production82 a direct effect on erythroid progenitors14 or by stimulation of RAS.83 Changes in the IGF-1 and Ac-SDKP levels also could alter erythropoiesis and thus contribute to PTE. AngII can modulate release of stimulatory factors (Epo, androgens, and IGF-1)26,41,84,85 as well as directly stimulate proliferation of erythroid progenitors. AngII appears to have a central role in excessive erythropoiesis of PTE (Fig 3). However, the AngII levels do not differ significantly between PTE and non-PTE patients.78 It appears that the major mechanism of the AngII effect stems from a hypersensitivity of erythroid progenitors to AngII through increased AT1R expression.50 Clinical studies provide further support for the AngII hypersensitivity as a mechanism of PTE. In particular, compared with patients without PTE, those with PTE require lower dozes of an ACE-I to achieve comparable decrements in hemoglobin concentration, hematocrit, and Epo levels.25 An ACE-I-mediated decrease of red blood cell survival was ruled out because no association was observed between the decrement in hematocrit and biochemical parameters of hemolysis.25,86,87

It is of interest that, similar to patients with PTE, patients with ESRD with polycythemia/erythrocytosis (mostly associated with acquired cystic kidney disease) showed no correlation between circulating Epo levels and hematocrit.69,70,77 Serum from the PTE patients stimulated BFU-E colonies, an effect that was partially reversed by anti-IGF-1 antibody. IGF-1 was thus implicated to have a crucial role in patients with ESRD with erythrocytosis and normal circulating Epo levels.
Similar to its effect on Epo synthesis, AngII can modulate production of IGF-1, as suggested by the lower circulating levels of IGF-1 after treatment of PTE patients with an ACE-I. The decrement appeared physiologically relevant because the change in IGF-1 levels correlated with the decrease in hematocrit. Recently, IGF-1 was shown to up-regulate expression of AT1R on smooth muscle cells. If IGF-1 has a corresponding effect on hematopoietic cells, it could accentuate the AngII-mediated stimulation of cellular proliferation in early erythropoiesis.

Role of Erythropoietin in Postrenal Transplant Erythrocytosis
Epo can play a role in the pathogenesis of PTE, although the exact mechanism remains controversial. Serum Epo levels in PTE patients widely differ. Although some investigators have found abnormally elevated Epo levels, others reported normal, decreased, or even undetectable Epo levels. Furthermore, serum Epo levels did not correlate with the size or number of BFU-E-derived colonies. These observations suggest that factors other than Epo could be instrumental for the development of PTE. Nonetheless, BFU-E progenitors from PTE patients have a greater sensitivity to Epo. Moreover, spontaneous growth of BFU-E colonies in the absence of Epo has been shown for some PTE patients.

Erythroid Progenitors in Postrenal Transplant Erythrocytosis
An increased number of BFU-E in patients with PTE suggests that production of early erythroid progenitors is inappropriately high. This effect could be a consequence of either increased levels of hematopoietic growth factors that might not be specific for erythropoiesis such as IGF-1, or by increased sensitivity of early erythroid progenitors to growth factors such as Epo. Furthermore, such a stimulation appears to be erythroid-specific because in patients with PTE, there is a decreased number of granulocyte/macrophage (GM) precursors. The AngII effect on erythropoiesis is confined predominantly to the early erythroid progenitors because AT1R expression is most prominent during early stages of erythropoiesis (ie, BFU-E) and decreases with maturation. A direct AngII stimulatory effect on proliferation of BFU-E was initially observed in semisolid culture after pretreatment of CD34+ cells in liquid media with AngII. The effect was reversible with ARB. Interestingly, addition of ACE-I to cell cultures also significantly inhibited the growth of BFU-E-derived cells, suggesting the presence of a paracrine or autocrine source of AngII within these cultures. When AT1R expression was measured in reference to IκBα, an ubiquitously expressed protein in BFU-E cells in culture for 7 to 14 days, the AT1R/IκBα ratios were significantly higher in PTE patients compared with renal transplant recipients without erythrocytosis or normal volunteers, and these ratios correlated with hematocrits. In these experiments, the AT1R was functional, as shown by an increase in intracellular calcium after stimulation of the cells with AngII. There was no correlation between serum AngII or Epo levels or plasma renin activity with the size or number of BFU-E-derived colonies. These findings suggest that overexpression of functional AT1R on early erythroid progenitors defines a pathophysiological role for RAS in the...
genesis of PTE. Because serum AngII levels do not differ significantly between PTE and non-PTE renal transplant patients, the effect of AngII on stimulation of other erythropoietic factors is likely limited or confined primarily to the hematopoietic compartment (e.g. bone marrow).

Epo-independent growth of BFU-E colonies from PTE patients has been observed in only some studies. A possible explanation of this spontaneous growth in the absence of “obligatory” Epo is the fact that erythroid progenitors can produce their own Epo. These quantities of Epo, although perhaps minimal, could be sufficient to prevent apoptosis in progenitors for which proliferation is sufficiently stimulated by non-Epo factors (i.e., AngII and IGF-1).

Therapy of Postrenal Transplant Erythrocytosis

Treatment of patients with PTE with drugs that suppress the RAS has virtually eliminated the need for therapeutic phlebotomy. The maximal reduction of hemoglobin levels usually manifests by 6 months after starting therapy with either an ACE-I or ARB. Some patients are exquisitely sensitive and could become severely anemic. Such therapy can inhibit erythropoiesis by several mechanisms:

1. ACE-I treatment can decrease circulating levels of Epo. However, baseline pretreatment serum Epo levels vary widely, and a primary role for Epo in PTE appears unlikely because changes in hematocrit during treatment with an ACE-I or ARB has been associated with variable, decreased, or unchanged serum Epo levels.

2. ACE-I therapy has also been associated with significant decreases in serum IGF-1 levels that were correlated with decrements in hematocrit. Interestingly, in renal transplant patients without PTE, ACE-I therapy increased IGF-1 levels, suggesting appropriate compensatory stimulation in an “AngII-deficient” state. The mechanisms of IGF-1 and AngII interactions in the regulation of erythropoiesis in PTE remain to be elucidated.

3. ACE-I therapy of renal transplant patients with or without PTE increased apoptosis of CD34+ cells within 2 to 3 weeks. The treatment effect peaked at approximately 3 to 6 weeks and disappeared 2 to 4 weeks after stopping ACE-I therapy. The apoptosis occurred concurrently with decrements in hematocrit that were more pronounced in the patients with PTE. This effect suggests that early erythroid progenitors can become AngII-dependent after renal transplantation.

4. ACE inactivates Ac-SDKP, a naturally occurring peptide that prevents recruitment of hematopoietic stem cells for production of red blood cells. This mechanism could explain, at least in part, the common clinical observation that ACE-Is are generally more effective than ARBs in decreasing the hematocrit in patients with PTE and are now the first choice for treatment.

PROPOSED MODEL FOR A ROLE OF ANGIOTENSIN IN ERYTHROPOIESIS AND POSTRENAL TRANSPLANT ERYTHROCYTOSIS

Under normal physiological conditions, multiple erythroid growth factors control erythropoiesis. Although proliferation of early erythroid progenitors can be modulated by many growth factors that are not unique to erythropoiesis (“non-Epo”), proliferation of the more mature progenitors appears to be controlled by Epo (Fig 1). This responsiveness can ensure a smooth transition from control of proliferation of immature cells by various non-Epo growth factors to more differentiated erythropoiesis fully regulated by Epo that is erythroid-specific. This erythroid specificity permits changes in Epo secretion to specifically alter red blood cell production without undesired parallel effects on proliferation of other cell types. Epo, with its production controlled by an oxygen sensor, could thus “fine tune” production of red blood cells by either enhanced stimulation of proliferation of more mature erythroid progenitors or by downregulation of Epo release that eliminates the excess of erythroid progenitors through apoptosis. We hypothesize that the autocrine Epo production provides the baseline “obligatory Epo requirement” for committed erythroid progenitors and protects against apoptosis of progenitors potentially already producing hemoglobin. Anemia of “Epo deficiency” resulting from inadequate secretion of Epo in response to appropriate oxygen sensing is common in patients with chronic renal insufficiency or ESRD. We speculate that this chronically inadequate Epo production is partially compensated by an increase in proliferation of early erythroid pro-
Furthermore, we propose that this effect is mediated by both “non-Epo” hematopoietic growth factors (eg, IGF-1) and an increase in sensitivity of erythroid progenitors to the erythropoiesis stimulating factors (eg, overexpression of AT1R) (Figs 4 and 5). This adjustment of erythropoiesis dynamics could first appear when the Epo response from failing kidneys proves insufficient to maintain oxygen delivery to peripheral tissues within the physiological range. This change probably becomes prominent with progressive loss of renal function over time. Because AngII, as well as IGF-1, plays an important role in early phases of erythropoiesis, in an “Epo-deficient” state (such as advanced renal failure), early erythropoiesis relies on “non-Epo” erythroid growth factors, including AngII and IGF-1. Indirect support for this hypothesis comes from observation that inhibition of ACE or blockade of AT1R in the “Epo-deficient” states decreases the hematocrit. In some “Epo-deficient” individuals, maintenance of sufficient erythropoiesis then becomes dependent on Epo as well as AngII, IGF-1, or both.

In the environment of chronic “Epo-deficient” erythropoiesis in ESRD, characterized by inefficient proliferation of the more mature, Epo-dependent progenitors and by increased production of less mature forms responding to more universal “non-Epo” hematopoietic growth factors, successful renal transplantation increases circulating Epo levels relatively quickly and permanently. We postulate that in renal transplant patients whose oxygen sensor-driven secretion of Epo does not decrease appropriately, PTE will develop (Fig 6). In these patients, the excessive proliferation of early erythroid progenitors could be driven so much by...
Fig 6. Proposed model for postrenal transplant erythrocytosis (PTE) pathogenesis. Under normal physiological conditions, there is a balance between requisite red blood cell production (eg, to replace lost red blood cells) and inhibitors of erythropoiesis (balance, left) and erythropoietic stimulatory factors such as erythropoietin (Epo), growth factors not unique to erythropoiesis (non-Epo), and erythroid progenitor sensitivity (PS) to these growth factors (balance, right). Sudden Epo deficiency (eg, after bilateral nephrectomy) causes an imbalance that decreases hemoglobin concentration (Hgb [g/dL]). We speculate that in chronic Epo-deficient states, Epo deficiency is partially offset by a compensatory increase in non-Epo erythroid growth factors and/or by an increase in progenitor sensitivity. Successful renal transplantation could restore a normal balance. However, if the sustained Epo increase is inappropriately high, the result is an excessive increase in hemoglobin concentration (ie, PTE). Blunting the compensatory increase in non-Epo factors and progenitor sensitivity with angiotensin-converting enzyme inhibitor or angiotensin II type 1 receptor blocker therapy restores normal balance. Similar rebalancing can occur spontaneously with time.

"non-Epo" growth factors and/or hypersensitivity of the progenitor cells to these factors that dampening or withdrawal of the stimulus (ie, treatment with an ACE-I) induces cellular apoptosis. Once proper oxygen sensing reduces Epo secretion and proliferation of the early erythroid progenitors normalizes, the PTE resolves.

According to this model, the increased effect of the "non-Epo" hematopoietic factors explains the variability in serum Epo levels in patients with PTE. The magnitude of this effect varies and could depend on the levels of "non-Epo" growth factors locally (eg, in bone marrow) or in the circulation and the degree of the hypersensitivity of erythroid progenitors. Epo-independent growth of BFU-E colonies from some patients with PTE could represent an extreme manifestation of this hypersensitivity.

SUMMARY

PTE is a common and potentially dangerous complication of successful renal transplantation, and AngII plays an important role in its pathogenesis. The AngII effect is probably related to its ability to augment proliferation of red blood cell...
progenitors through stimulation of Epo-driven signal transduction pathways, as well as the ability of erythroid progenitors to overexpress AT1R under certain conditions (“Epo-deficient” states). AngII can also stimulate erythropoiesis indirectly (eg, through increased secretion of Epo or IGF-1). We speculate that some of the growth factors modulating proliferation of early hematopoietic progenitors such as AngII continue to exert this effect during the early stages of erythropoiesis, and that this effect serves as a compensatory mechanism in “Epo-deficient” states. We postulate that in some patients after successful renal transplantation, Epo secretion is inappropriately high for the degree of this “non-Epo”-mediated compensation and that this impaired regulation culminates in erythrocytosis. Inhibition of AngII as one of the principal “non-Epo” erythroid factors effectively decreases red blood cell production in clinical conditions with an expected compensatory augmentation of erythropoiesis modulated by “non-Epo” factors. Fortunately, there is an effective remedy: both ACE-I and ARBs are safe and effective, and their use should be considered standard therapy for patients with PTE.

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