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## TARGETING THE INTRAGRAFT MICROENVIRONMENT AND THE DEVELOPMENT OF CHRONIC ALLOGRAFT REJECTION\*

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### Abstract

In this review, we discuss a paradigm whereby changes in the intragraft microenvironment promote or sustain the development of chronic allograft rejection. A key feature of this model involves changes in the microvasculature including a) endothelial cell (EC) destruction, and b) EC proliferation, both of which result from alloimmune leukocyte- and/or alloantibody-induced responses. These changes in the microvasculature likely create abnormal blood flow patterns and thus promote local tissue hypoxia. Another feature of the chronic rejection microenvironment involves the overexpression of *vascular endothelial growth factor* (VEGF). VEGF stimulates EC activation and proliferation and it has potential to sustain inflammation via direct interactions with leukocytes. In this manner, VEGF may promote ongoing tissue injury. Finally, we review how these events can be targeted therapeutically using mTOR inhibitors. EC activation and proliferation as well as VEGF-VEGFR interactions require PI-3K/Akt/mTOR intracellular signaling. Thus, agents that inhibit this signaling pathway within the graft may also target the progression of chronic rejection and thus promote long-term graft survival.

### Keywords

Endothelial Cell; Microvascular Injury; Angiogenesis; Vascular Endothelial Growth Factor; Hypoxia; Allograft Rejection; Chronic Allograft Rejection; Allograft Vasculopathy

### Overview

A large body of literature indicates that pathological changes within the microvasculature are characteristic of acute and chronic allograft rejection. Here, we will review recent

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concepts indicating that microvascular endothelial cells (EC) participate in all aspects of rejection, from their initial encounter with leukocytes to the angiogenesis response that is characteristic of the chronic inflammatory healing process. Endothelial cell (EC) activation responses are initiated within allografts as a result of the biological effects of cytokines released from resident macrophages in response to hypoxia and ischemia-reperfusion injury [1]. Persistent EC activation occurs in association with chronic rejection, a cell-mediated immune response characterized by repeated episodes of acute inflammation and associated attempts at repair [2, 3]. These proinflammatory EC activation responses include the induced expression of adhesion molecules, chemokines and MHC class I and II molecules on the cell surface of donor graft EC [1, 4, 5]. Activated endothelial cells mediate the recruitment and infiltration of leukocytes within the allograft tissue [1, 2, 6-8], promote leukocyte activation directly and facilitate functional differentiation of transmigrating leukocytes [9, 10]. This includes the reactivation of T cells to produce Th1 [11], Th2 [12], or Th17 [13] cytokines and the ability of EC to mediate the differentiation of monocytes into professional antigen presenting cells (APCs) [5, 14]. Collectively, these events create a pathological microenvironment within the graft that we propose both initiates and sustains the rejection process. Thus, targeting events within the microvasculature have potential to inhibit the chronic rejection process. A discussion of the molecular basis for EC-dependent recruitment and leukocyte activation has been recently reviewed in depth elsewhere [1, 7, 15] and is beyond the scope of this review. Here, we discuss other key aspects of the microenvironment that are integral to chronic allograft rejection

## The Initiation of the Pathological Intra-graft Microenvironment

It is widely accepted that acute and chronic rejection is initiated and sustained by the recipient's immunological response to donor antigen, coordinated by CD4<sup>+</sup> T cells and other cell types including CD8<sup>+</sup> T cells, B cells and macrophages [5, 16-18]. The alloimmune response involves multiple cell types and complex issues including the expansion of effector cells [19, 20], clonal size [21-23], the mode of allorecognition ("direct" or "indirect" pathways) [16, 24-26], the development of alloantibody [27-30] and immunoregulation [31-36]. However, donor-specific alloantibodies, cytokines as well as growth factors must target the graft, and notably they must injure vascular EC in order to mediate graft destruction. Alloimmune-dependent targeting of EC can cause direct injury to the graft and result in local tissue hypoxia, but it can also promote EC activation and proliferation, which is associated with leukocyte infiltration. We have recently proposed that initial ischemia-reperfusion injury and the later immune response creates an intra-graft microenvironment that fosters the development of chronic allograft rejection [37, 38]. In this paradigm, three major elements within the microenvironment contribute to the rejection process. The first is direct injury to the graft EC that results from ischemia-reperfusion injury, cellular and humoral alloimmune targeting. The second is EC proliferation that occurs as a characteristic consequence of delayed type hypersensitivity [3], and is mediated by mononuclear cell infiltrates and the production of local cytokines and growth factors. Both events (direct targeting and EC proliferation) serve to create abnormal microvascular blood flow patterns and thus, local tissue hypoxia [37, 39-41], and they precede endothelial-to-mesenchymal transition (EndMT), whereby abnormal EC may dedifferentiate into fibroblasts [42, 43]. The third key event is the overexpression of *vascular endothelial growth factor* (VEGF), which is both pro-inflammatory and pro-angiogenic, and is thus a key molecule in the development of the chronic rejection microenvironment. Below, we will focus on these three aspects of this paradigm and we will discuss how inflammation results in angiogenesis, and how VEGF plays a key role in the pathological intra-graft microenvironment.

## Overlapping Nature of Angiogenesis and Chronic Inflammation in Allografts

As discussed above, early ischemia-reperfusion as well as cellular and humoral targeting of the graft EC results in profound injury to the microvasculature [15, 27, 38, 44, 45]. The loss of microvascular integrity impairs the delivery of oxygen and nutrients to interstitial cells, which in turn contributes to local tissue ischemia, and cell death (Figure 1 and [44, 46, 47]). Indeed, the degree of injury and microvascular EC loss at early times post transplantation has been reported to be predictive of the development of interstitial fibrosis, tubular atrophy (IFTA) as well as later chronic rejection following kidney transplantation [48]. Pharmacologic therapy that augments protective signaling in EC and maintains microvascular integrity at early times post transplantation has potential to improve long-term graft survival [44, 49]. These studies suggest that the lack of early protective and/or homeostatic repair responses within the capillary bed will be associated with the subsequent development of chronic rejection [38].

However, inflammatory infiltrates also mediate a process of leukocyte-induced angiogenesis [3, 50-52]. EC proliferation and the creation of new blood vessels is necessary for normal wound healing and physiologic tissue repair following acute injury [3,53]. It has also been found to be associated with many chronic inflammatory disease states [50, 51, 54] including chronic allograft rejection [38, 45]. Importantly, the chronic inflammatory neoangiogenesis response can result in a disorganized pattern of blood vessels [38, 41, 45, 55] that are described to be irregular in size with chaotic branching patterns [39]. Thus, the response has potential to create associated abnormalities in blood flow throughout the inflamed tissue [38, 39, 41]. Once present within allografts [41], we suggest that some areas of the graft may have increased blood flow while other areas have sluggish blood flow that can result in patchy areas of tissue hypoxia [38, 41]. Thus, once angiogenesis is present within a graft, it is likely associated with local hypoxia and thus, it has high potential to support the progression of tissue injury/disease, [3, 45, 50, 52, 53].

In the course of an immune inflammatory reaction, vascular repair processes are regulated by the local expression and the relative balance and function of pro- and anti-angiogenesis factors. Monocytes, which are characteristic of chronic inflammation, are well established to mediate angiogenesis [56, 57]. The molecular basis for monocyte-EC interactions and the resultant EC proliferative response is understood to involve the secretion of several pro-angiogenic mediators including VEGF [57], TNF- [56], TGF- and nitric oxide [58, 59]. Some of these latter factors have been found to function in part by stimulating the production of VEGF [56, 57]. Also, it has also been found that activated T cells are a major source of angiogenesis factors, including VEGF [60, 61]. Collectively, these findings support the hypothesis that VEGF is a key mediator of the inflammatory angiogenesis reaction [45]. In addition, other factors, such as chemokines, that are produced in association with inflammatory responses have the ability to regulate EC proliferation [62]. Thus, it is not surprising that during chronic inflammation, EC proliferative responses and angiogenesis are overlapping and interactive processes [3, 50, 52-54].

During inflammation, the excessive production of VEGF and related angiogenesis factors occurs both temporally and spatially in association with leukocytic infiltrates [45, 55, 63-65]. EC proliferation and angiogenesis has been reported to occur within the intimal proliferating lesion of allograft vasculopathy [66-68]. Aberrant angiogenesis has also been observed in association with bronchiolitis obliterans, the pathophysiological correlate of chronic lung rejection [41, 69]. Once EC proliferation occurs within an allograft, its effect on blood flow patterns and local tissue hypoxia has potential to induce VEGF expression and thus, the inflammatory angiogenesis response [38, 39, 41], and in turn, local tissue

hypoxia results in an amplification of VEGF expression and the inflammatory angiogenesis response. As such, over time, the angiogenesis response and VEGF expression provide amplification loops to support an abnormal intragraft microenvironment that sustains the progression of chronic allograft rejection [38, 39, 41, 45, 70, 71].

## Overexpression of Vascular Endothelial Growth Factor (VEGF): A Key Intragraft Pathological Feature of Allograft Rejection

The major stimulus for VEGF expression is hypoxia [39, 70, 71], but it can be induced by several cytokines, including IL-1, TNF, and IL-6 [56, 72, 73]. In addition, the ligation of CD40 on EC and monocytes by CD154 (CD40 ligand, expressed by activated platelets and T cells) is potent to induce VEGF expression [61, 74]. Since many of these VEGF-inducing factors are present within allografts at different times post transplantation, it is not surprising that VEGF-dependent biological responses are a characteristic feature of the pathological intragraft microenvironment. For instance, as discussed above, during rejection local tissue hypoxia may induce the overexpression of VEGF (illustrated in Figure 1). In addition, VEGF is delivered into the local intragraft microenvironment by inflammatory infiltrates indicating a high likelihood that it will be associated with the rejection process. Indeed, VEGF is not only expressed, but it has emerged as a key mediator of both acute and chronic allograft rejection [45, 65, 75]. Once, present within the graft microenvironment, VEGF classically functions as an angiogenesis factor [45, 46, 76], but increasing evidence suggests that it may also act as a potent proinflammatory cytokine [45, 77, 78]. To this end, VEGF may be functional to mediate the progression of chronic disease [45, 50, 51, 63, 69, 79-81].

VEGF functions as a proinflammatory cytokine in part related to its ability to induce the expression of adhesion molecules and chemokines in EC [63, 77, 82, 83] and also to its function as a direct leukocyte chemoattractant [63, 78, 81, 84]. The VEGF receptors Flt-1 (VEGF receptor 1) and neuropilin-1 are expressed by human monocytes and APCs [78, 79, 85], and VEGF-VEGFR interactions function to elicit monocyte activation responses as well as chemotactic activity [78, 79]. In addition, several recent studies have indicated that T cell subsets express Flt-1, KDR (VEGF receptor 2) and neuropilin-1 [80, 84, 86, 87], and VEGF-VEGFR interactions promote lymphocyte chemotaxis *in vitro* and *in vivo* [63, 80, 81, 84]. In models of acute rejection, antibodies to VEGF or to Flt-1 and KDR prolong graft survival [63, 88]. In models of chronic rejection, the overexpression of VEGF within cardiac allografts mobilizes bone marrow derived monocyte/macrophages and accelerates the development of allograft vasculopathy [89]. In humanized SCID mouse models, blockade of VEGF or VEGFR's inhibits the development of acute rejection as well as the development of allograft vasculopathy [63, 80, 81]. In part these effects of VEGF-VEGFR blockade have been reported to be associated with the inhibition of the intragraft accumulation of T cells [80, 81]. Interestingly, VEGFR-expressing T cells have been found to accumulate within rejecting human allografts *in vivo* [80, 81], suggesting that locally expressed VEGF within allografts may interact with VEGFRs expressed on subsets of effector T cells to facilitate lymphocyte chemotaxis. Consistent with this possibility, anti-VEGF and anti-KDR inhibit the transmigration of CD4<sup>+</sup> and CD8<sup>+</sup> T cells across activated EC *in vitro*. These observations demonstrate that once VEGF is overexpressed within an allograft, the microenvironment promotes inflammation, chronic rejection and allograft vasculopathy [89].

Indeed, consistent with this model, Pilmore *et al* [65] observed that VEGF expression was most striking in association with CD68<sup>+</sup> monocyte/macrophage infiltrates within the interstitium of human renal allografts. Torry *et al* [75] found that the expression of VEGF was confined to areas with monocyte/macrophage infiltrates in cardiac allografts, and we observed that its expression was prominent in association with inflammatory cell infiltrates

[64]. In addition, we have found that high levels of intragraft VEGF correlated with the development of both acute and chronic cardiac allograft rejection, and that persistent expression identified patients who were at high risk for the development of cardiac allograft vasculopathy/chronic rejection [64]. Levels of VEGF may also increase significantly in the serum and urine of patients with cardiac and renal allograft rejection [90, 91], and human recipients of transplants with genotypes encoding high VEGF production are at increased risk for the development of allograft rejection [92, 93]. Taken together, these observations support the hypothesis that VEGF is mechanistic to elicit events that sustain the pathological intragraft microenvironment.

## The Mammalian Target of Rapamycin (mTOR) Signaling Pathway In Vascular Endothelial Cells

Activation of mTOR plays a central role in EC activation, survival and proliferation [94-97]. Indeed, targeting mTOR in EC with rapamycin is well established to inhibit EC survival, to reduce angiogenesis in experimental models [94, 96] and to delay wound healing *in vivo* [98, 99]. Targeting mTOR also reduces leukocyte-induced angiogenesis in association with inflammation [45, 97]. In this next section, we focus our discussion on how the mTOR signaling pathway functions to promote the activation, survival and proliferation of graft vascular EC. mTOR inhibitors represent the first-in-kind anti-EC therapeutics that are used in clinical practice following transplantation. Here, we show how they target the maintenance and progression of the chronic rejection intragraft microenvironment (illustrated in Figure 1 and Figure 2).

mTOR is a serine/threonine kinase present in two distinct protein complexes [100]. mTOR complex-1 (mTORC1) is composed of mTOR, regulatory-associated protein of mTOR (raptor) and mammalian lethal with SEC13 protein 8 (mLST8) [101, 102], and functions to couple signals generated from growth factors, cytokines, nutrients and amino acids to cell growth and proliferation [103]. mTORC2 is composed of mTOR, rapamycin-insensitive companion of mTOR (riCTOR), mLST8, proline-rich protein 5 (ProtOR) and mitogen-activated protein kinase-associated protein 1 (mSin1) [104, 105], and it functions to phosphorylate and activate AGC kinase family members such as Akt, SGK and PKC [106, 107].

Receptor mediated signals initially lead to the activation of the phosphoinositide-3 kinase (PI-3K)/Akt and the Raf/MEK/MAPK signaling pathways, which phosphorylate and inactivate TSC2 [108, 109], resulting in the activation of the small GTPase Rheb, which in turn, directly activates mTORC1 [110, 111]. In addition, growth factors can increase mTORC1 activity through TSC2-independent mechanisms, including a pathway that involves Akt-dependent phosphorylation of PRAS40, which removes the inhibitory effect of TSC on mTORC1 activity [112]. In response to upstream stimuli, mTORC1 ultimately regulates cell growth, proliferation and protein synthesis through the phosphorylation of two well-characterized substrates 4E-BP1 and S6K1 [113].

In contrast, little is known about the upstream regulation of mTORC2 assembly and activation [114]. To date, it has been reported that growth factors activate mTORC2 in a PI-3K dependent manner through a mechanism that involves the association of mTORC2 to ribosomes [115, 116]. It is however well established that mTORC2 mediates Akt activity, which in turn activates mTORC1. This signaling response, via mTORC1, can also initiate a feedback loop to regulate mTORC2 activity through interactions with rictor [114]. Thus, mTOR kinase activity is closely interrelated with the activity of the Akt kinase and crosstalk between both mTOR complexes (see Figure 2).

## mTOR Signaling and The Pathological Intragraft Environment

As discussed above, the expression of VEGF within allografts has potential to promote and sustain a microenvironment that fosters the development of chronic rejection. To this end, both mTORC1 and mTORC2 are signaling intermediaries between inflammation and the production of VEGF. For instance, the ligation of CD40 on EC by CD154 stimulates VEGF transcriptional activation in an mTORC2-dependent manner [74]. In addition, VEGF is secreted at high levels in cells with increased mTORC1 activity [117]. At the molecular level, mTORC1 enhances the expression of HIF-1, a transcription factor that is known to induce the expression of VEGF ([118] and Figure 2). HIF-1 activity is also induced by hypoxia, suggesting several mechanisms whereby VEGF expression will be induced by cytokines and/or hypoxia *in vivo* within allografts.

mTOR inhibitors are also potent to target EC activation and proliferative responses, key features of chronic rejection. The molecular mechanisms implicated in the inhibition of EC proliferation by rapamycin have only been partially characterized, but include a reduction in cyclin D1 expression as well as inhibition of Akt-induced responses [95, 119]. Akt is an evolutionarily conserved serine/threonine kinase, which is well known to mediate cell survival and resistance to apoptosis [107, 120]. Three different Akt isoforms are present in mammals and they share ~80% homology in amino acid sequence. Activation of Akt requires phosphorylation of the Thr308 and Ser473 amino acid residues by PDK1 and mTORC2 respectively [106]. Following activation, the Akt kinase regulates numerous signals including mTORC1 that result in cell survival or the inhibition of apoptosis. For example, Akt-activity induces the phosphorylation of BAD, which prevents its binding to pro-apoptotic Bcl-XL [121]. Also, Akt-mediated phosphorylation of the forkhead family of transcription factors including Foxo1 and Foxo3a mediates their sequestration in the cytoplasm, which prevents the induction of pro-apoptotic genes by these factors [122]. The proliferative effects of Akt are mediated by p21, p27 and cyclin D1 expression as well as through several additional effects that result from mTORC1 activation [123].

Many of these observations suggest that Akt is implicated in EC responses pertinent to the development of the chronic rejection [45, 97]. In addition, it is important to note that Akt is also functional in EC-dependent mechanisms of proinflammation. For instance, TNF-induced expression of the chemokine MCP-1 is reduced by pharmacological inhibitors of PI-3K/Akt signaling [124]. Also, Akt-mediated signals induce the expression of the T cell chemoattractant chemokine IP-10 (also called CXCL10) in EC [83]. Collectively, these reports suggest that targeting Akt/mTOR signals in EC has potential to inhibit activation responses, including chemokine expression and the inducible expression of VEGF, and to inhibit EC proliferative responses.

### Targeting mTOR signaling: Therapeutic Considerations

Cellular rejection is associated with cytokine- and growth factor-mediated responses in EC that include activation of the mTOR signaling pathway [1, 97, 98]. In addition humoral immune responses characterized by the production of donor specific alloantibodies contribute to vascular injury and chronic rejection by inducing EC activation and proliferation [27, 125]. The basis for alloantibody-mediated responses in EC is an area of current research [27], but recent studies indicate that binding is associated with the activation of mTOR [125]. We suggest that this response relates to physiological induction of protective genes, but as discussed above, it also results in the induction of proinflammatory chemokines as well as VEGF that together serve to enhance alloimmune-dependent injury to the graft (Figure 1). Thus, it is possible that mTOR signaling inhibitors

have potential to attenuate intracellular cascades within the graft, so that the EC response is limited and/or that the EC phenotype remains quiescent.

Rapamycin and its pharmacological analogues, collectively called rapalogs, are currently used therapeutically following transplantation [97-99] as well as in other disease states [100, 103] to inhibit the mTOR signaling pathway in multiple cell types. The effect of rapalogs on the inhibition of chronic rejection has been evaluated in different experimental models [126]. In addition, following heart transplantation in humans, evidence suggests that the addition of rapalogs to the immunosuppression regimen reduces the development of allograft vasculopathy [127, 128]. Interestingly, rapalogs not only limit the development of allograft vasculopathy but also reduce the progression of established lesions. While these reports are encouraging, some experimental studies have demonstrated that blocking mTOR with rapalogs might be suboptimal in the targeting of activation responses. First, rapalogs have no direct effect on mTORC2, which mediates VEGF expression [74]. Second, rapalogs only partially inhibit mTORC1 activity resulting in limited inhibition of EC proliferation [129]. Since the induced expression of VEGF is proposed to be central to the chronic rejection microenvironment, more efficient targeting of both mTORC2 and mTORC1 might be of theoretical benefit to prevent the progression of chronic rejection.

Rapamycin binds to FKBP-12 to form a complex with the FRB domain of mTOR, resulting in inhibition of activation [100]. This complex inhibits mTORC1, but it has no direct effect on mTORC2, presumably because the FRB domain of mTOR is not accessible to the rapamycin-FKBP-12 complex. Nevertheless, in some cell types, including EC, prolonged treatment with rapamycin also blocks mTORC2 activity by inhibiting the *de novo* formation of the complex [95]. We suggest that this effect might in part be related to the ability of high intracellular concentrations of rapamycin to saturate the binding of mTOR, such that less is available for assembly as mTORC2 [95, 97]. Alternatively, conformational changes may inhibit mTORC2 activity. Nevertheless, this effect of rapamycin results in an inhibition of the activity of the Akt kinase, which is the downstream effector of mTORC2 activity [95, 107, 130].

A new class of drugs have been recently developed that block the mTOR kinase domain by acting as ATP-competitive inhibitors [129, 131]. Compared to rapalogs, ATP-competitive inhibitors of mTOR target both mTORC1 and mTORC2. Initial studies have demonstrated that their ability to inhibit EC proliferation and survival are greater than rapalogs [131], and they also inhibit VEGF production more effectively. These differences indicate that ATP-competitive inhibitors of mTOR have promise as novel therapeutics in the future.

Finally, it is important to note that mTOR is integral to a complex of signaling networks and crosstalk among signaling cascades. Therefore, agents that inhibit mTOR activity also influence other signaling pathways that are involved in cell proliferation and survival [100, 107]. For instance, the inhibition of mTOR by rapalogs or ATP-competitive inhibitors induces the activation of the MEK/MAPK signaling pathway [131]. As MEK/MAPK generates proliferative and survival signals it is likely that co-incident activation of these this pathway by mTOR inhibitors limits their biological effects. Thus, in the future it is possible that the combination of classes of mTOR inhibitors with MEK inhibitors may be therapeutic to target intracellular signals more efficiently than either treatment alone [131].

## Summary and Conclusion

In this review, we have defined EC-based events such as those mediated by cellular and humoral immunity that contribute to chronic rejection. Once EC are injured, changes in the microcirculation result in local areas of tissue hypoxia within the graft. In addition,



cytokines and growth factors within the microenvironment stimulate EC activation, which sustains the inflammatory reaction further amplifying the local injury response. EC proliferation and angiogenesis may also lead to abnormal blood flow patterns within the graft to create local tissue ischemia, which serves to sustain the progression of chronic rejection. We propose that local tissue hypoxia together with the overexpression of VEGF and VEGF-VEGFR interactions are central determinants of chronic rejection. Since activation of the mTOR/Akt signaling pathway plays a central role in the EC activation and proliferation in response to cellular and alloimmune targeting, it is possible that the use of rapalogs will inhibit intragraft EC-dependent events pertinent to the progression of chronic rejection. Overall, this review provides insight into the intragraft microenvironment as a novel paradigm that highlights mechanisms of chronic rejection. We suggest that this paradigm also has potential to identify areas for future therapeutic intervention to inhibit chronic rejection and promote long-term survival following transplantation.

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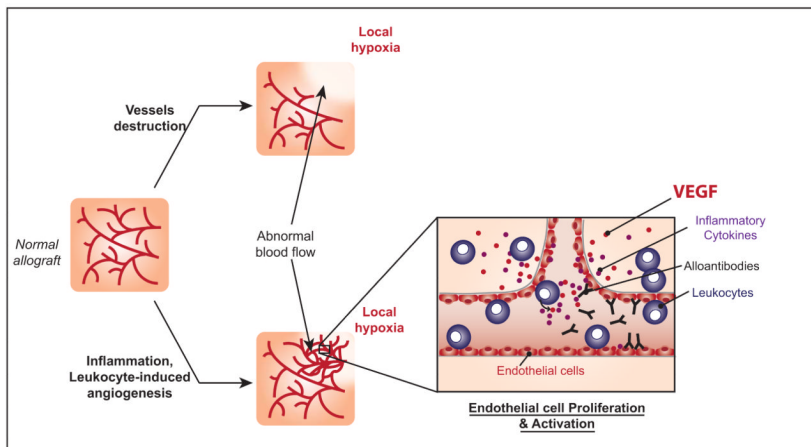
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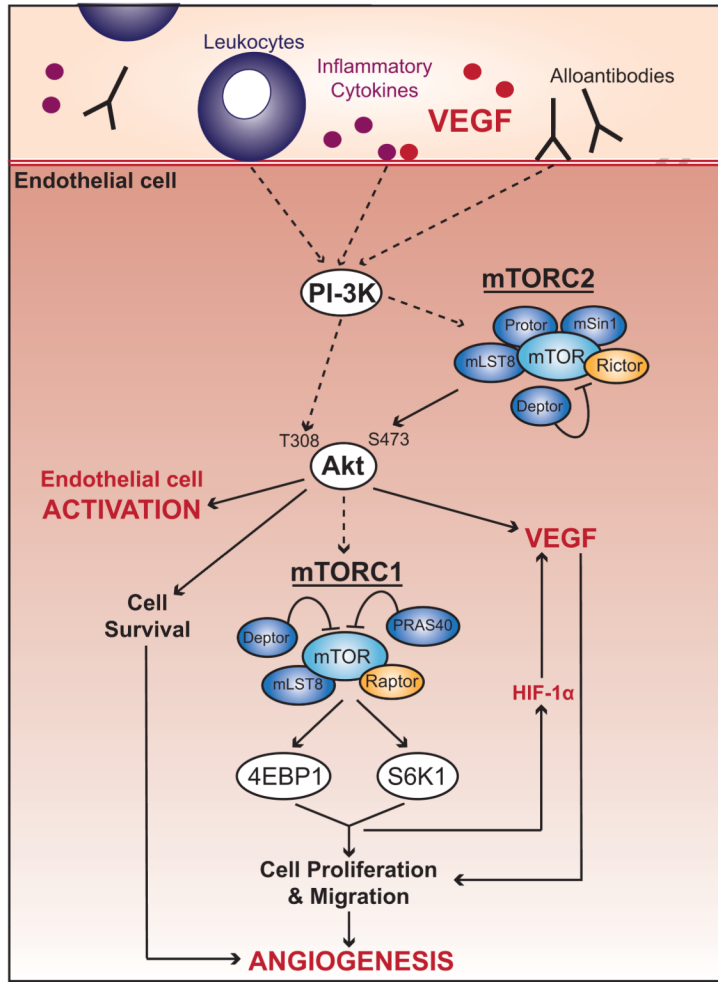
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**Figure 1. Cartoon illustrating the interplay between alloimmunity, the intragraft microvasculature and chronic allograft rejection**

Following transplantation, early alloimmune inflammatory targeting of the donor graft vascular endothelium results in the destruction of microvessels and local tissue hypoxia and injury. In addition, inflammatory responses may also stimulate endothelial cell (EC) activation and proliferation, and a leukocyte-induced angiogenesis reaction. In part, this response results from the delivery of cytokines and pro-angiogenic factors including *Vascular Endothelial Growth Factor* (VEGF) into the graft by infiltrating leukocytes. The pathological leukocyte-induced EC proliferation results in changes in the microvasculature, including the formation of abnormal networks of capillaries and chaotic or sluggish blood flow patterns that have also been shown to result in local tissue hypoxia. Thus, local tissue hypoxia, and hypoxia-inducible genes (such as VEGF) may sustain ongoing tissue damage. We thus propose that the pathological intragraft microenvironment that sustains chronic rejection results from both acute targeting of EC, as well as from EC proliferation/angiogenesis.



**Figure 2. Cartoon illustrating mTOR signaling pathway that may function to mediate the pathological inflammatory microenvironment**  
 During allograft rejection, alloantibodies, inflammatory cytokines and growth factors mediate activation of the mTOR signaling pathway. Assembly of the mTORC2 complex facilitates the phosphorylation and activation of Akt, which alone is sufficient to mediate EC activation responses and the transcriptional activation of VEGF. pAkt is well established to facilitate cell survival responses but also mediates growth, proliferation and migration and protein synthesis via mTORC1-dependent responses including two substrates S6K1 and 4EBP1. In EC these events are critical for proliferation and the neoangiogenesis reaction. mTORC1-mediated signals also enhance the expression of HIF-1 (a hypoxia response transcription factor), which induces the expression of VEGF. These signals are regulated by cell intrinsic proteins including PRAS40 and Deptor, and can be targeted therapeutically by rapalogs.