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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 Intragraft 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 $CD4^+$ T cells and other cell types including $CD8^+$ 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 intragraft 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 intragraft 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 $CD4^+$ and $CD8^+$ 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 $CD68^+$ 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 central to the chronic rejection miroenvironment, 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 raplogs [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. Ee 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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References

1. Pober JS, Sessa WC. Evolving functions of endothelial cells in inflammation. Nat Rev Immunol. 2007; 7(10):803. [PubMed: 17893694]
2. Denton MD, Davis SF, Baum MA, Melter M, Reinders ME, Exeni A, Samsonov DV, Fang J, Ganz P, Briscoe DM. The role of the graft endothelium in transplant rejection: evidence that endothelial activation may serve as a clinical marker for the development of chronic rejection. Pediatr Transplant. Nov.2000 4(4):252. [PubMed: 11079263]
3. Cotran, RS. Inflammation and repair. In: Cotran, RS.; Kumar, V.; Robbins, SL., editors. Pathologic basis of disease. W.B. Saunders; Philidelphia: 1994. p. 51
4. Kreisel D, Krupnick AS, Gelman AE, Engels FH, Popma SH, Krasinskas AM, Balsara KR, Szeto WY, Turka LA, Rosengard BR. Non-hematopoietic allograft cells directly activate CD8+ T cells and trigger acute rejection: an alternative mechanism of allorecognition. Nat Med. Mar.2002 8(3): 233. [PubMed: 11875493]
5. Briscoe DM, Sayegh MH. A rendezvous before rejection: where do T cells meet transplant antigens? Nat Med. Mar.2002 8(3):220. [PubMed: 11875489]
6. Libby P, Pober JS. Chronic rejection. Immunity. 2001; 14(4):387. [PubMed: 11336684]
7. Valujskikh A, Heeger PS. Emerging roles of endothelial cells in transplant rejection. Curr Opin Immunol. 2003; 15(5):493. [PubMed: 14499255]
8. Pober JS, Orosz CG, Rose ML, Savage COS. Can graft endothelial cells initiate a host anti-graft immune response? Transplantation. 1996; 61:343. [PubMed: 8610337]
9. Denton MD, Geehan C, Alexander SI, Sayegh MH, Briscoe DM. Endothelial cells modify the costimulatory capacity of transmigrating leukocytes and promote CD28-mediated CD4+ T cell alloactivation. J Exp Med. 1999; 190:555. [PubMed: 10449526]
10. Briscoe DM. Recent insights into the role(s) of the endothelium in allorecognition. Graft. 1999; 2:261.
11. Hughes CC, Savage CO, Pober JS. Endothelial cells augment T cell interleukin 2 production by a contact-dependent mechanism involving CD2/LFA-3 interaction. J Exp Med. 1990; 171(5):1453. [PubMed: 1692079]
12. Ma W, Pober JS. Human endothelial cells effectively costimulate cytokine production by, but not differentiation of, naive CD4+ T cells. J Immunol. 1998; 161:2158. [PubMed: 9725207]
13. Taflin C, Favier B, Baudhuin J, Savenay A, Hemon P, Bensussan A, Charron D, Glotz D, Mooney N. Human endothelial cells generate Th17 and regulatory T cells under inflammatory conditions. Proc Natl Acad Sci U S A. 2011; 108(7):2891. [PubMed: 21282653]

14. Randolph GJ, Beaulieu S, Lebecque S, Steinman RM, Muller WA. Differentiation of monocytes into dendritic cells in a model of transendothelial trafficking. Science. 1998; 282:480. [PubMed: 9774276]
15. Vos IH, Briscoe DM. Endothelial injury: cause and effect of alloimmune inflammation. Transpl Infect Dis. 2002; 4(3):152. [PubMed: 12421461]
16. Sayegh MH. Why do we reject a graft? Role of indirect allorecognition in graft rejection. Kidney Int. Nov.1999 56(5):1967. [PubMed: 10571983]
17. Rothstein DM, Sayegh MH. T-cell costimulatory pathways in allograft rejection and tolerance. Immunol Rev. 2003; 196:85. [PubMed: 14617200]
18. Sayegh MH, Turka LA. The role of T-cell costimulatory activation pathways in transplant rejection. N Engl J Med. Jun 18.1998 338(25):1813. [PubMed: 9632449]
19. Lakkis FG, Arakelov A, Konieczny BT, Inoue Y. Immunologic 'ignorance' of vascularized organ transplants in the absence of secondary lymphoid tissue. Nat Med. Jun.2000 6(6):686. [PubMed: 10835686]
20. Lakkis FG. Where is the alloimmune response initiated? Am J Transplant. 2003; 3(3):241. [PubMed: 12614275]
21. Li Y, Li XC, Zheng XX, Wells AD, Turka LA, Strom TB. Blocking both signal 1 and signal 2 of T-cell activation prevents apoptosis of alloreactive T cells and induction of peripheral allograft tolerance. Nat Med. Nov.1999 5(11):1298. [PubMed: 10545997]
22. Wells AD, Li XC, Li Y, Walsh MC, Zheng XX, Wu Z, Nunez G, Tang A, Sayegh M, Hancock WW, Strom TB, Turka LA. Requirement for T-cell apoptosis in the induction of peripheral transplantation tolerance. Nat Med. Nov.1999 5(11):1303. [PubMed: 10545998]
23. Li XC, Strom TB, Turka LA, Wells AD. T cell death and transplantation tolerance. Immunity. Apr. 2001 14(4):407. [PubMed: 11336686]
24. Sayegh MH, Carpenter CB. Role of indirect allorecognition in allograft rejection. Int Rev Immunol. 1996; 13(3):221. [PubMed: 8782743]
25. Jiang S, Herrera O, Lechler RI. New spectrum of allorecognition pathways: implications for graft rejection and transplantation tolerance. Curr Opin Immunol. 2004; 16(5):550. [PubMed: 15341998]
26. Lechler RI, Lombardi G, Batchelor JR, Reinsmoen N, Bach FH. The molecular basis of alloreactivity. Immunol Today. Mar.1990 11(3):83. [PubMed: 2186745]
27. Zhang X, Reed EF. Effect of antibodies on endothelium. Am J Transplant. 2009; 9(11):2459. [PubMed: 19775314]
28. Sis B, Halloran PF. Endothelial transcripts uncover a previously unknown phenotype: C4d-negative antibody-mediated rejection. Curr Opin Organ Transplant. 2010; 15(1):42. [PubMed: 20009933]
29. Baldwin WM 3rd, Halushka MK, Valujskikh A, Fairchild RL. B cells in cardiac transplants: From clinical questions to experimental models. Semin Immunol. 2012; 24(2):122. [PubMed: 21937238]
30. Bradley JA, Baldwin WM, Bingaman A, Ellenrieder C, Gebel HM, Glotz D, Kirk AD. Antibody-mediated rejection--an ounce of prevention is worth a pound of cure. Am J Transplant. 2011; 11(6):1131. [PubMed: 21645250]
31. Li XC, Rothstein DM, Sayegh MH. Costimulatory pathways in transplantation: challenges and new developments. Immunol Rev. 2009; 229(1):271. [PubMed: 19426228]
32. Brusko TM, Putnam AL, Bluestone JA. Human regulatory T cells: role in autoimmune disease and therapeutic opportunities. Immunol Rev. 2008; 223:371. [PubMed: 18613848]
33. Wing K, Sakaguchi S. Regulatory T cells exert checks and balances on self tolerance and autoimmunity. Nat Immunol. 2010; 11(1):7. [PubMed: 20016504]
34. Wood KJ, Sakaguchi S. Regulatory T cells in transplantation tolerance. Nat Rev Immunol. 2003; 3(3):199. [PubMed: 12658268]
35. Lechler RI, Ng WF, Camara NO. Infectious tolerance? Mechanisms and implications. Transplantation. 2001; 72(8 Suppl):S29. [PubMed: 11888153]
36. Salama AD, Remuzzi G, Harmon WE, Sayegh MH. Challenges to achieving clinical transplantation tolerance. J Clin Invest. Oct.2001 108(7):943. [PubMed: 11581293]

37. Bruneau S, Woda CB, Daly KP, Boneschansker L, Jain NG, Kochupurakkal N, Contreras AG, Seto T, Briscoe DM. Key Features of the Intragraft Microenvironment that Determine Long-Term Survival Following Transplantation. Front Immunol. 2012; 3:54. [PubMed: 22566935]
38. Contreras AG, Briscoe DM. Every allograft needs a silver lining. J Clin Invest. 2007; 117(12): 3645. [PubMed: 18060023]
39. Goel S, Duda DG, Xu L, Munn LL, Boucher Y, Fukumura D, Jain RK. Normalization of the vasculature for treatment of cancer and other diseases. Physiol Rev. 2011; 91(3):1071. [PubMed: 21742796]
40. Jain RK. Taming vessels to treat cancer. Sci Am. 2008; 298(1):56. [PubMed: 18225696]
41. Babu AN, Murakawa T, Thurman JM, Miller EJ, Henson PM, Zamora MR, Voelkel NF, Nicolls MR. Microvascular destruction identifies murine allografts that cannot be rescued from airway fibrosis. J Clin Invest. 2007; 117(12):3774. [PubMed: 18060031]
42. Zeisberg EM, Tarnavski O, Zeisberg M, Dorfman AL, McMullen JR, Gustafsson E, Chandraker A, Yuan X, Pu WT, Roberts AB, Neilson EG, Sayegh MH, Izumo S, Kalluri R. Endothelial-to-mesenchymal transition contributes to cardiac fibrosis. Nat Med. 2007; 13(8):952. [PubMed: 17660828]
43. Medici D, Shore EM, Lounev VY, Kaplan FS, Kalluri R, Olsen BR. Conversion of vascular endothelial cells into multipotent stem-like cells. Nat Med. 2010; 16(12):1400. [PubMed: 21102460]
44. Aydin Z, van Zonneveld AJ, de Fijter JW, Rabelink TJ. New horizons in prevention and treatment of ischaemic injury to kidney transplants. Nephrol Dial Transplant. 2007; 22(2):342. [PubMed: 17132706]
45. Reinders ME, Rabelink TJ, Briscoe DM. Angiogenesis and endothelial cell repair in renal disease and allograft rejection. J Am Soc Nephrol. 2006; 17(4):932. [PubMed: 16481411]
46. Mayer G. Capillary rarefaction, hypoxia, VEGF and angiogenesis in chronic renal disease. Nephrol Dial Transplant. 2011; 26(4):1132. [PubMed: 21330358]
47. Fine LG, Norman JT. Chronic hypoxia as a mechanism of progression of chronic kidney diseases: from hypothesis to novel therapeutics. Kidney Int. 2008; 74(7):867. [PubMed: 18633339]
48. Steegh FM, Gelens MA, Nieman FH, van Hooff JP, Cleutjens JP, van Suylen RJ, Daemen MJ, van Heurn EL, Christiaans MH, Peutz-Kootstra CJ. Early loss of peritubular capillaries after kidney transplantation. J Am Soc Nephrol. 2011; 22(6):1024. [PubMed: 21566051]
49. Nakao A, Choi AM, Murase N. Protective effect of carbon monoxide in transplantation. J Cell Mol Med. 2006; 10(3):650. [PubMed: 16989726]
50. Folkman, J.; Brem, H. Angiogenesis and inflammation. In: Gallin, JI.; Goldstein, IM.; Synderman, R., editors. Inflammation: Basic principles and clinical correlates. Raven Press; New York: 1992. p. 821
51. Ferrara N, Alitalo K. Clinical applications of angiogenic growth factors and their inhibitors. Nat Med. Dec.1999 5(12):1359. [PubMed: 10581076]
52. Auerbach R, Sidky YA. Nature of the stimulus leading to lymphocyte-induced angiogenesis. J Immunol. Aug.1979 123(2):751. [PubMed: 37274]
53. Majno G. Chronic inflammation: links with angiogenesis and wound healing. Am J Pathol. 1998; 153:1035. [PubMed: 9777935]
54. Ezaki T, Baluk P, Thurston G, La Barbara A, Woo C, McDonald DM. Time course of endothelial cell proliferation and microvascular remodeling in chronic inflammation. Am J Pathol. 2001; 158(6):2043. [PubMed: 11395382]
55. Moulton KS, Melder RJ, Dharnidharka VR, Hardin-Young J, Jain RK, Briscoe DM. Angiogenesis in the huPBL-SCID model of human transplant rejection. Transplantation. 1999; 67(12):1626. [PubMed: 10401773]
56. Leibovich SJ, Polverini PJ, Shepard HM, Wiseman DM, Shively V, Nuseir N. Macrophage-induced angiogenesis is mediated by tumour necrosis factor-alpha. Nature. Oct 15-21.1987 329(6140):630. 329(6140. [PubMed: 2443857]
57. Polverini PJ. Role of the macrophage in angiogenesis-dependent diseases. EXS. 1997; 79:11. [PubMed: 9002218]

58. Wiseman DM, Polverini PJ, Kamp DW, Leibovich SJ. Transforming growth factor-beta (TGF beta) is chemotactic for human monocytes and induces their expression of angiogenic activity. Biochem Biophys Res Commun. Dec 15.1988 157(2):793. 157(2. [PubMed: 2462419]
59. Leibovich SJ, Polverini PJ, Fong TW, Harlow LA, Koch AE. Production of angiogenic activity by human monocytes requires an L-arginine/nitric oxide-synthase-dependent effector mechanism. Proc Natl Acad Sci U S A. May 10.1994 91(10):4190. [PubMed: 7514298]
60. Freeman MR, Schneck FX, Gagnon ML, Corless C, Soker S, Niknejad K, Peoples GE, Klagsbrun M. Peripheral blood T lymphocytes and lymphocytes infiltrating human cancers express vascular endothelial growth factor: a potential role for T cells in angiogenesis. Cancer Res. Sep 15.1995 55(18):4140. [PubMed: 7545086]
61. Melter M, Reinders ME, Sho M, Pal S, Geehan C, Denton MD, Mukhopadhyay D, Briscoe DM. Ligation of CD40 induces the expression of vascular endothelial growth factor by endothelial cells and monocytes and promotes angiogenesis in vivo. Blood. 2000; 96(12):3801. [PubMed: 11090063]
62. Belperio JA, Keane MP, Arenberg DA, Addison CL, Ehlert JE, Burdick MD, Strieter RM. CXC chemokines in angiogenesis. J Leukoc Biol. Jul.2000 68(1):1. [PubMed: 10914483]
63. Reinders ME, Sho M, Izawa A, Wang P, Mukhopadhyay D, Koss KE, Geehan CS, Luster AD, Sayegh MH, Briscoe DM. Proinflammatory functions of vascular endothelial growth factor in alloimmunity. J Clin Invest. 2003; 112(11):1655. [PubMed: 14660742]
64. Reinders ME, Fang JC, Wong W, Ganz P, Briscoe DM. Expression patterns of vascular endothelial growth factor in human cardiac allografts: association with rejection. Transplantation. 2003; 76(1): 224. [PubMed: 12865814]
65. Pilmore HL, Eris JM, Painter DM, Bishop GA, McCaughan GW. Vascular endothelial growth factor expression in human chronic renal allograft rejection. Transplantation. 1999; 67(6):929. [PubMed: 10199746]
66. Tanaka H, Sukhova GK, Libby P. Interaction of the allogeneic state and hypercholesterolemia in arterial lesion formation in experimental cardiac allografts. Arterioscler Thromb. May.1994 14(5): 734. [PubMed: 8172851]
67. Denton MD, Magee C, Melter M, Dharnidharka VR, Sayegh MH, Briscoe DM. TNP-470, an angiogenesis inhibitor, attenuates the development of allograft vasculopathy. Transplantation. 2004; 78(8):1218. [PubMed: 15502723]
68. Atkinson C, Southwood M, Pitman R, Phillpotts C, Wallwork J, Goddard M. Angiogenesis occurs within the intimal proliferation that characterizes transplant coronary artery vasculopathy. J Heart Lung Transplant. 2005; 24(5):551. [PubMed: 15896752]
69. Belperio JA, Weigt SS, Fishbein MC, Lynch JP 3rd. Chronic lung allograft rejection: mechanisms and therapy. Proc Am Thorac Soc. 2009; 6(1):108. [PubMed: 19131536]
70. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature. Oct 29.1992 359(6398):843. [PubMed: 1279431]
71. Mukhopadhyay D, Tsiokas L, Zhou XM, Foster D, Brugge JS, Sukhatme VP. Hypoxic induction of human vascular endothelial growth factor expression through c-Src activation. Nature. 1995; 375:577. [PubMed: 7540725]
72. Amano K, Okigaki M, Adachi Y, Fujiyama S, Mori Y, Kosaki A, Iwasaka T, Matsubara H. Mechanism for IL-1 beta-mediated neovascularization unmasked by IL-1 beta knock-out mice. J Mol Cell Cardiol. 2004; 36(4):469. [PubMed: 15081307]
73. Huang SP, Wu MS, Shun CT, Wang HP, Lin MT, Kuo ML, Lin JT. Interleukin-6 increases vascular endothelial growth factor and angiogenesis in gastric carcinoma. J Biomed Sci. 2004; 11(4):517. [PubMed: 15153787]
74. Dormond O, Contreras AG, Meijer E, Datta D, Flynn E, Pal S, Briscoe DM. CD40-induced signaling in human endothelial cells results in mTORC2- and Akt-dependent expression of vascular endothelial growth factor in vitro and in vivo. J Immunol. 2008; 181(11):8088. [PubMed: 19018001]
75. Torry RJ, Labarrere CA, Torry DS, Holt VJ, Faulk WP. Vascular endothelial growth factor expression in transplanted human hearts. Transplantation. 1995; 60(12):1451. [PubMed: 8545873]

76. Kang DH, Hughes J, Mazzali M, Schreiner GF, Johnson RJ. Impaired angiogenesis in the remnant kidney model: II. Vascular endothelial growth factor administration reduces renal fibrosis and stabilizes renal function. J Am Soc Nephrol. 2001; 12(7):1448. [PubMed: 11423573]
77. Melder RJ, Koenig GC, Witwer BP, Safabakhsh N, Munn LL, Jain RK. During angiogenesis, vascular endothelial growth factor and basic fibroblast growth factor regulate natural killer cell adhesion to tumor endothelium. Nat Med. Sep.1996 2(9):992. [PubMed: 8782456]
78. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marme D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. Blood. Apr 15.1996 87(8):3336. [PubMed: 8605350]
79. Chapoval SP, Lee CG, Tang C, Keegan AD, Cohn L, Bottomly K, Elias JA. Lung vascular endothelial growth factor expression induces local myeloid dendritic cell activation. Clin Immunol. 2009; 132(3):371. [PubMed: 19553159]
80. Edelbauer M, Datta D, Vos IH, Basu A, Stack MP, Reinders ME, Sho M, Calzadilla K, Ganz P, Briscoe DM. Effect of vascular endothelial growth factor and its receptor KDR on the transendothelial migration and local trafficking of human T cells in vitro and in vivo. Blood. 2010; 116(11):1980. [PubMed: 20538805]
81. Zhang J, Silva T, Yarovinsky T, Manes TD, Tavakoli S, Nie L, Tellides G, Pober JS, Bender JR, Sadeghi MM. VEGF blockade inhibits lymphocyte recruitment and ameliorates immune-mediated vascular remodeling. Circ Res. 2010; 107(3):408. [PubMed: 20538685]
82. Kim I, Moon SO, Kim SH, Kim HJ, Koh YS, Koh GY. Vascular endothelial growth factor expression of intercellular adhesion molecule 1 (ICAM-1), vascular cell adhesion molecule 1 (VCAM-1), and E-selectin through nuclear factor-kappa B activation in endothelial cells. J Biol Chem. Mar 9.2001 276(10):7614. [PubMed: 11108718]
83. Boulday G, Haskova Z, Reinders ME, Pal S, Briscoe DM. Vascular endothelial growth factor-induced signaling pathways in endothelial cells that mediate overexpression of the chemokine IFN-gamma-inducible protein of 10 kDa in vitro and in vivo. J Immunol. 2006; 176(5):3098. [PubMed: 16493069]
84. Basu A, Hoerning A, Datta D, Edelbauer M, Stack MP, Calzadilla K, Pal S, Briscoe DM. Cutting edge: Vascular endothelial growth factor-mediated signaling in human CD45RO+ CD4+ T cells promotes Akt and ERK activation and costimulates IFN-gamma production. J Immunol. 2010; 184(2):545. [PubMed: 20008289]
85. Romeo PH, Lemarchandel V, Tordjman R. Neuropilin-1 in the immune system. Adv Exp Med Biol. 2002; 515:49. [PubMed: 12613542]
86. Sarris M, Andersen KG, Randow F, Mayr L, Betz AG. Neuropilin-1 expression on regulatory T cells enhances their interactions with dendritic cells during antigen recognition. Immunity. 2008; 28(3):402. [PubMed: 18328743]
87. Suzuki H, Onishi H, Wada J, Yamasaki A, Tanaka H, Nakano K, Morisaki T, Katano M. VEGFR2 is selectively expressed by FOXP3high CD4+ Treg. Eur J Immunol. 2010; 40(1):197. [PubMed: 19902430]
88. Sho M, Akashi S, Kanehiro H, Hamada K, Kashizuka H, Ikeda N, Nomi T, Kuzumoto Y, Tsurui Y, Yoshiji H, Wu Y, Hicklin DJ, Briscoe DM, Nakajima Y. Function of the vascular endothelial growth factor receptors Flt-1 and Flk-1/KDR in the alloimmune response in vivo. Transplantation. 2005; 80(6):717. [PubMed: 16210956]
89. Lemstrom KB, Krebs R, Nykanen AI, Tikkanen JM, Sihvola RK, Aaltola EM, Hayry PJ, Wood J, Alitalo K, Yla-Herttuala S, Koskinen PK. Vascular endothelial growth factor enhances cardiac allograft arteriosclerosis. Circulation. May 28.2002 105(21):2524. [PubMed: 12034660]
90. Abramson LP, Pahl E, Huang L, Stellmach V, Rodgers S, Mavroudis C, Backer CL, Arensman RM, Crawford SE. Serum vascular endothelial growth factor as a surveillance marker for cellular rejection in pediatric cardiac transplantation. Transplantation. Jan 15.2002 73(1):153. [PubMed: 11792998]
91. Peng W, Chen J, Jiang Y, Shou Z, Chen Y, Wang H. Acute renal allograft rejection is associated with increased levels of vascular endothelial growth factor in the urine. Nephrology (Carlton). 2008; 13(1):73. [PubMed: 18199108]

92. Shahbazi M, Fryer AA, Pravica V, Brogan IJ, Ramsay HM, Hutchinson IV, Harden PN. Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. J Am Soc Nephrol. Jan.2002 13(1):260. [PubMed: 11752046]

93. Girnita DM, Brooks MM, Webber SA, Burckart GJ, Ferrell R, Zdanowicz G, DeCroo S, Smith L, Chinnock R, Canter C, Addonizio L, Bernstein D, Kirklin JK, Ranganathan S, Naftel D, Girnita AL, Zeevi A. Genetic polymorphisms impact the risk of acute rejection in pediatric heart transplantation: a multi-institutional study. Transplantation. 2008; 85(11):1632. [PubMed: 18551071]

94. Guba M, von Breitenbuch P, Steinbauer M, Koehl G, Flegel S, Hornung M, Bruns CJ, Zuelke C, Farkas S, Anthuber M, Jauch KW, Geissler EK. Rapamycin inhibits primary and metastatic tumor growth by antiangiogenesis: involvement of vascular endothelial growth factor. Nat Med. 2002; 8(2):128. [PubMed: 11821896]

95. Dormond O, Madsen JC, Briscoe DM. The Effects of mTOR-Akt Interactions on Anti-apoptotic Signaling in Vascular Endothelial Cells. J Biol Chem. 2007; 282(32):23679. [PubMed: 17553806]

96. Phung TL, Ziv K, Dabydeen D, Eyiah-Mensah G, Riveros M, Perruzzi C, Sun J, Monahan-Earley RA, Shiojima I, Nagy JA, Lin MI, Walsh K, Dvorak AM, Briscoe DM, Neeman M, Sessa WC, Dvorak HF, Benjamin LE. Pathological angiogenesis is induced by sustained Akt signaling and inhibited by rapamycin. Cancer Cell. 2006; 10(2):159. [PubMed: 16904613]

97. Contreras AG, Dormond O, Edelbauer M, Calzadilla K, Hoerning A, Pal S, Briscoe DM. mTOR-understanding the clinical effects. Transplant Proc. 2008; 40(10 Suppl):S9. [PubMed: 19100913]

98. Weir MR, Diekmann F, Flechner SM, Lebranchu Y, Mandelbrot DA, Oberbauer R, Kahan BD. mTOR inhibition: the learning curve in kidney transplantation. Transpl Int. 2010; 23(5):447. [PubMed: 20136784]

99. Kahan BD. Fifteen years of clinical studies and clinical practice in renal transplantation: reviewing outcomes with de novo use of sirolimus in combination with cyclosporine. Transplant Proc. 2008; 40(10 Suppl):S17. [PubMed: 19100899]

100. Zoncu R, Efeyan A, Sabatini DM. mTOR: from growth signal integration to cancer, diabetes and ageing. Nat Rev Mol Cell Biol. 2011; 12(1):21. [PubMed: 21157483]

101. Kim DH, Sarbassov DD, Ali SM, King JE, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. mTOR interacts with raptor to form a nutrient-sensitive complex that signals to the cell growth machinery. Cell. 2002; 110(2):163. [PubMed: 12150925]

102. Kim DH, Sarbassov DD, Ali SM, Latek RR, Guntur KV, Erdjument-Bromage H, Tempst P, Sabatini DM. GbetaL, a positive regulator of the rapamycin-sensitive pathway required for the nutrient-sensitive interaction between raptor and mTOR. Mol Cell. 2003; 11(4):895. [PubMed: 12718876]

103. Dazert E, Hall MN. mTOR signaling in disease. Curr Opin Cell Biol. 2011; 23(6):744. [PubMed: 21963299]

104. Jacinto E, Loewith R, Schmidt A, Lin S, Ruegg MA, Hall A, Hall MN. Mammalian TOR complex 2 controls the actin cytoskeleton and is rapamycin insensitive. Nat Cell Biol. 2004; 6(11):1122. [PubMed: 15467718]

105. Sarbassov DD, Ali SM, Kim DH, Guertin DA, Latek RR, Erdjument-Bromage H, Tempst P, Sabatini DM. Rictor, a novel binding partner of mTOR, defines a rapamycin-insensitive and raptor-independent pathway that regulates the cytoskeleton. Curr Biol. 2004; 14(14):1296. [PubMed: 15268862]

106. Sarbassov DD, Guertin DA, Ali SM, Sabatini DM. Phosphorylation and regulation of Akt/PKB by the rictor-mTOR complex. Science. 2005; 307(5712):1098. [PubMed: 15718470]

107. Jacinto E, Lorberg A. TOR regulation of AGC kinases in yeast and mammals. Biochem J. 2008; 410(1):19. [PubMed: 18215152]

108. Potter CJ, Pedraza LG, Xu T. Akt regulates growth by directly phosphorylating Tsc2. Nat Cell Biol. 2002; 4(9):658. [PubMed: 12172554]

109. Ballif BA, Roux PP, Gerber SA, MacKeigan JP, Blenis J, Gygi SP. Quantitative phosphorylation profiling of the ERK/p90 ribosomal S6 kinase-signaling cassette and its targets, the tuberous sclerosis tumor suppressors. Proc Natl Acad Sci U S A. 2005; 102(3):667. [PubMed: 15647351]

110. Inoki K, Li Y, Xu T, Guan KL. Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Dev. 2003; 17(15):1829. [PubMed: 12869586]

111. Tee AR, Manning BD, Roux PP, Cantley LC, Blenis J. Tuberous sclerosis complex gene products, Tuberin and Hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. Curr Biol. 2003; 13(15):1259. [PubMed: 12906785]

112. Vander Haar E, Lee SI, Bandhakavi S, Griffin TJ, Kim DH. Insulin signalling to mTOR mediated by the Akt/PKB substrate PRAS40. Nat Cell Biol. 2007; 9(3):316. [PubMed: 17277771]

113. Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. Nat Rev Mol Cell Biol. 2009; 10(5):307. [PubMed: 19339977]

114. Dibble CC, Asara JM, Manning BD. Characterization of Rictor phosphorylation sites reveals direct regulation of mTOR complex 2 by S6K1. Mol Cell Biol. 2009; 29(21):5657. [PubMed: 19720745]

115. Zinzalla V, Stracka D, Oppliger W, Hall MN. Activation of mTORC2 by association with the ribosome. Cell. 2011; 144(5):757. [PubMed: 21376236]

116. Gan X, Wang J, Su B, Wu D. Evidence for direct activation of mTORC2 kinase activity by phosphatidylinositol 3,4,5-trisphosphate. J Biol Chem. 2011; 286(13):10998. [PubMed: 21310961]

117. El-Hashemite N, Walker V, Zhang H, Kwiatkowski DJ. Loss of Tsc1 or Tsc2 induces vascular endothelial growth factor production through mammalian target of rapamycin. Cancer Res. 2003; 63(17):5173. [PubMed: 14500340]

118. Hudson CC, Liu M, Chiang GG, Otterness DM, Loomis DC, Kaper F, Giaccia AJ, Abraham RT. Regulation of hypoxia-inducible factor 1alpha expression and function by the mammalian target of rapamycin. Mol Cell Biol. 2002; 22(20):7004. [PubMed: 12242281]

119. Vinals F, Chambard JC, Pouyssegur J. p70 S6 kinase-mediated protein synthesis is a critical step for vascular endothelial cell proliferation. J Biol Chem. 1999; 274(38):26776. [PubMed: 10480882]

120. Franke TF. PI3K/Akt: getting it right matters. Oncogene. 2008; 27(50):6473. [PubMed: 18955974]

121. Downward J. How BAD phosphorylation is good for survival. Nat Cell Biol. 1999; 1(2):E33. [PubMed: 10559890]

122. Brunet A, Bonni A, Zigmond MJ, Lin MZ, Juo P, Hu LS, Anderson MJ, Arden KC, Blenis J, Greenberg ME. Akt promotes cell survival by phosphorylating and inhibiting a Forkhead transcription factor. Cell. 1999; 96(6):857. [PubMed: 10102273]

123. Zhou BP, Liao Y, Xia W, Spohn B, Lee MH, Hung MC. Cytoplasmic localization of p21Cip1/WAF1 by Akt-induced phosphorylation in HER-2/neu-overexpressing cells. Nat Cell Biol. 2001; 3(3):245. [PubMed: 11231573]

124. Murao K, Ohyama T, Imachi H, Ishida T, Cao WM, Namihira H, Sato M, Wong NC, Takahara J. TNF-alpha stimulation of MCP-1 expression is mediated by the Akt/PKB signal transduction pathway in vascular endothelial cells. Biochem Biophys Res Commun. 2000; 276(2):791. [PubMed: 11027549]

125. Jindra PT, Jin YP, Rozengurt E, Reed EF. HLA class I antibody-mediated endothelial cell proliferation via the mTOR pathway. J Immunol. 2008; 180(4):2357. [PubMed: 18250445]

126. Raichlin E, Kushwaha SS. Proliferation signal inhibitors and cardiac allograft vasculopathy. Curr Opin Organ Transplant. 2008; 13(5):543. [PubMed: 19060540]

127. Eisen HJ, Tuzcu EM, Dorent R, Kobashigawa J, Mancini D, Valantine-von Kaeppler HA, Starling RC, Sorensen K, Hummel M, Lind JM, Abeywickrama KH, Bernhardt P. Everolimus for the prevention of allograft rejection and vasculopathy in cardiac-transplant recipients. N Engl J Med. 2003; 349(9):847. [PubMed: 12944570]

128. Arora S, Ueland T, Wennerblom B, Sigurdadottir V, Eiskjaer H, Botker HE, Ekmehag B, Jansson K, Mortensen SA, Saunamaki K, Simonsen S, Gude E, Bendz B, Solbu D, Aukrust P, Gullestad L. Effect of everolimus introduction on cardiac allograft vasculopathy--results of a randomized, multicenter trial. Transplantation. 2011; 92(2):235. [PubMed: 21677600]

129. Thoreen CC, Kang SA, Chang JW, Liu Q, Zhang J, Gao Y, Reichling LJ, Sim T, Sabatini DM, Gray NS. An ATP-competitive mammalian target of rapamycin inhibitor reveals rapamycin-resistant functions of mTORC1. J Biol Chem. 2009; 284(12):8023. [PubMed: 19150980]

130. Sarbassov DD, Ali SM, Sengupta S, Sheen JH, Hsu PP, Bagley AF, Markhard AL, Sabatini DM. Prolonged rapamycin treatment inhibits mTORC2 assembly and Akt/PKB. Mol Cell. 2006; 22(2):159. [PubMed: 16603397]

131. Dormond-Meuwly A, Roulin D, Dufour M, Benoit M, Demartines N, Dormond O. The inhibition of MAPK potentiates the anti-angiogenic efficacy of mTOR inhibitors. Biochem Biophys Res Commun. 2011; 407(4):714. [PubMed: 21439267]

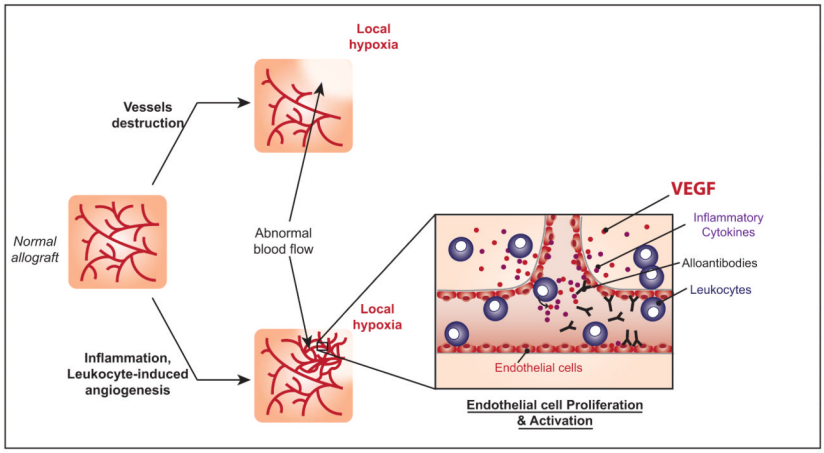

Figure 1. Cartoon illustrating the interplay between alloimmunity, the intragraft microvasculature and chronic allograft rejection

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.

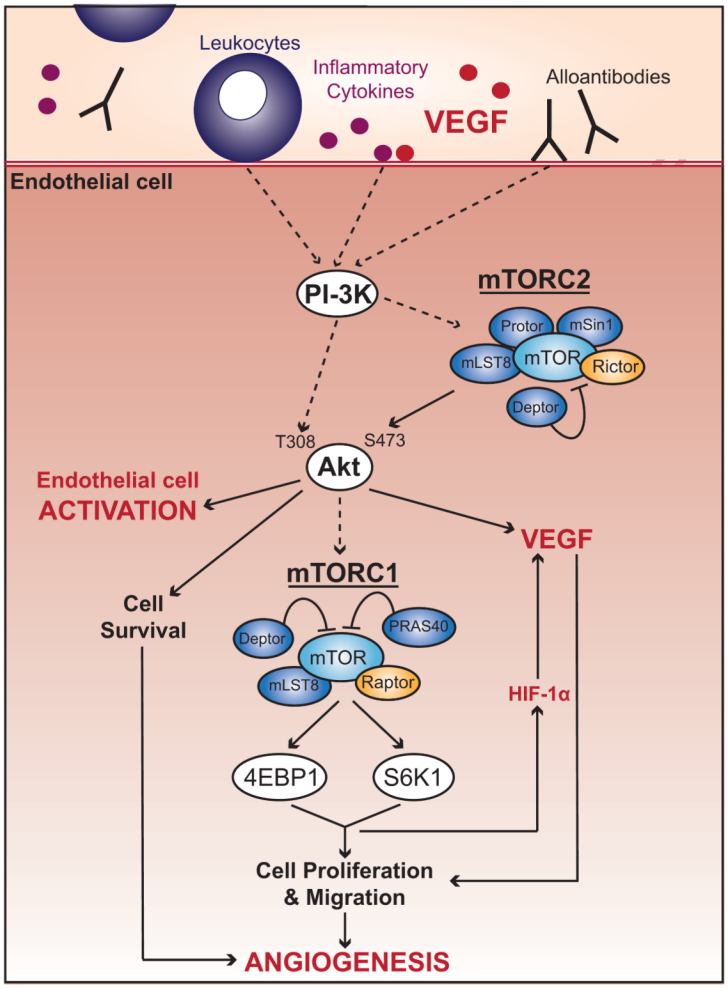

Figure 2. Cartoon illustrating mTOR signaling pathway that may function to mediate the pathological inflammatory microenvironment

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.