Inhibition of the mTORC Pathway in the Antiphospholipid Syndrome

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ABSTRACT


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BACKGROUND

Although thrombosis is considered the cardinal feature of the antiphospholipid syndrome, chronic vascular lesions are common, particularly in patients with life-threatening complications. In patients who require transplantation, vascular lesions often recur. The molecular pathways involved in the vasculopathy of the antiphospholipid syndrome are unknown, and adequate therapies are lacking.

METHODS

We used double immunostaining to evaluate pathway activation in the mammalian target of rapamycin complex (mTORC) and the nature of cell proliferation in the vessels of patients with primary or secondary antiphospholipid syndrome nephropathy. We also evaluated autopsy specimens from persons who had catastrophic antiphospholipid syndrome. The molecular pathways through which antiphospholipid antibodies modulate the mTORC pathway were evaluated in vitro, and potential pharmacologic inhibitors were also tested in vitro. Finally, we studied the effect of sirolimus in kidney-transplant recipients with the antiphospholipid syndrome.

RESULTS

The vascular endothelium of proliferating intrarenal vessels from patients with antiphospholipid syndrome nephropathy showed indications of activation of the mTORC pathway. In cultured vascular endothelial cells, IgG antibodies from patients with the antiphospholipid syndrome stimulated mTORC through the phosphatidyl­inositol 3-kinase (PI3K)–AKT pathway. Patients with antiphospholipid syndrome nephropathy who required transplantation and were receiving sirolimus had no recurrence of vascular lesions and had decreased vascular proliferation on biopsies as compared with patients with antiphospholipid antibodies who were not receiving sirolimus. Among 10 patients treated with sirolimus, 7 (70%) had a functioning renal allograft 144 months after transplantation versus 3 of 27 untreated patients (11%). Activation of mTORC was also found in the vessels of autopsy specimens from patients with catastrophic antiphospholipid syndrome.

CONCLUSIONS

Our results suggest that the mTOR pathway is involved in the vascular lesions associated with the antiphospholipid syndrome. (Funded by INSERM and others.)
The antiphospholipid syndrome is an autoimmune disease characterized by the presence of circulating antiphospholipid antibodies that result in vascular thrombosis and obstetrical complications. The syndrome may be isolated or may occur in association with autoimmune disorders, such as systemic lupus erythematosus. Thrombotic events represent the major complication of the antiphospholipid syndrome, and to date, long-term anticoagulation has been the only treatment shown to reduce vascular complications. However, that regimen does not prevent organ deterioration and death in high-risk patients, particularly those in whom catastrophic antiphospholipid syndrome develops.

In addition to the thrombotic complications, vascular cellular infiltrates and fibrosis of the intima and media develop in affected patients. Although initially reported in patients with the antiphospholipid syndrome accompanied by nephropathy, such lesions have now been observed in the coronary, carotid, and mesenteric arteries of patients with life-threatening complications of the antiphospholipid syndrome (e.g., myocardial infarction, stroke, or mesenteric ischemia). In patients with renal involvement, there is often progression to end-stage renal failure. We previously reported that antiphospholipid syndrome nephropathy may recur after renal transplantation, with resultant allograft loss. The molecular pathways involved in these lesions are unknown.

The mammalian target of rapamycin (mTOR) is a kinase that integrates a variety of signaling pathways to regulate cellular growth, proliferation, and survival. The enzyme is a component of two functionally distinct complexes, mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2); the pathways activated by these complexes exhibit cross-talk (i.e., the stimulation of one can initiate signaling in the other). Studies in both experimental models and patients undergoing arterial angioplasty indicate that mTORC2 plays a crucial role in the vascular stenosis that results from mechanical endothelial injury. Sirolimus, which inhibits the mTORC pathway, prevents or minimizes the formation of neointima after injury, an observation that is consistent with these findings. Consequently, mTOR inhibitors such as sirolimus are currently used to prevent reactive arterial stenosis after coronary stenting. Such observations, taken together, led us to hypothesize that activation of the mTORC pathway plays a role in the vascular changes that are characteristic of antiphospholipid syndrome nephropathy.

Methods

Patients and Data Collection

We studied four groups of patients: patients with primary antiphospholipid syndrome and associated nephropathy observed on renal biopsy, patients with secondary antiphospholipid syndrome and associated nephropathy superimposed on systemic lupus nephritis, patients with systemic lupus nephritis in the absence of the antiphospholipid syndrome, and patients with samples of normal renal tissue from kidneys that had been resected because of tumor (controls). Renal function was determined at the time of biopsy in the four groups of patients.

In addition, a previously described cohort of kidney-transplant recipients was studied (Table 1). Between January 2000 and December 2009, a total of 1359 kidney transplantations were performed at our institution. Among the patients receiving these transplants, we identified 37 recipients with antiphospholipid antibodies. We compared this group with 74 recipients without such antibodies, using a nested case–control method. Patients were followed until January 2012 (mean ± SD follow-up, 82 ± 35 months). All unsensitized transplant recipients were randomly assigned according to physician preference to one of two post-transplantation immunosuppressive regimens, one consisting of glucocorticoids, calcineurin inhibitors, and purine inhibitors and the other consisting of glucocorticoids, sirolimus, and purine inhibitors. In the group without antiphospholipid antibodies, 18 of 74 patients received sirolimus, and in the group with antiphospholipid antibodies, 10 of 37 patients received sirolimus. Surveillance kidney biopsies were performed at 3 and 12 months after transplantation in all patients with a functioning allograft, unless there was a medical contraindication; the glomerular filtration rate (GFR) was also determined at those time points.

We also evaluated vascular specimens from two groups of deceased patients: 4 patients who died from catastrophic antiphospholipid syndrome, and 4 patients who died from complications of systemic lupus erythematosus but did not have evidence of the antiphospholipid syndrome.

Our institutional review board approved the study, and written informed consent was obtained.
Renal, Histologic, and Functional Studies

The ability of antiphospholipid antibodies to modulate the mTORC pathway was studied in vitro with the use of IgG antibodies obtained from 12 patients with the antiphospholipid syndrome and 14 healthy volunteers. Vascular lesions were scored as described previously. In the patients with kidney transplants, renal lesions were also scored with the use of the Banff classification. Phosphorylation of AKT and S6RP was evaluated with the use of immunohistochemical, immunofluorescence, and colocalization experiments. Renal function was determined on the basis of serum creatinine levels or iohexol clearance. A detailed description of the methods used is provided in the Supplementary Appendix, available with the full text of this article at NEJM.org.

Statistical Analysis

Differences among the experimental groups were evaluated with the use of an analysis of variance (ANOVA) model, followed by the Tukey–Kramer test when findings with the ANOVA model were significant. When only two groups were compared, the Mann–Whitney test was used.

Table 1. Demographic and Clinical Characteristics of the Study Patients.*

<table>
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<tr>
<th>Characteristic</th>
<th>Controls (N = 10)</th>
<th>Patients with APS (N = 12)</th>
<th>Patients with SLE (N = 45)</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Without APS (N = 25)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>With APS (N = 20)</td>
</tr>
<tr>
<td>Patients without kidney transplants</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean age at biopsy — yr</td>
<td>48±13</td>
<td>43±24</td>
<td>28±12</td>
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<tr>
<td>Female sex — no. (%)</td>
<td>3 (33)</td>
<td>9 (75)</td>
<td>17 (68)</td>
</tr>
<tr>
<td>Estimated GFR at time of biopsy — ml/min/1.73 m²</td>
<td>88±12</td>
<td>46±5</td>
<td>47±15</td>
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<tr>
<td>Lupus anticoagulant — no. (%)</td>
<td>0</td>
<td>12 (100)</td>
<td>0</td>
</tr>
<tr>
<td>Anti-β₂-glycoprotein I antibodies — no. (%)</td>
<td>0</td>
<td>10 (83)</td>
<td>0</td>
</tr>
<tr>
<td>Anticardiolipin antibodies — no. (%)</td>
<td>0</td>
<td>10 (83)</td>
<td>0</td>
</tr>
<tr>
<td>Patients with kidney transplants</td>
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<td></td>
</tr>
<tr>
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<td>47±14</td>
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<tr>
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<td>34 (46)</td>
<td>13 (48)</td>
<td>4 (40)</td>
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<td>Age at time of end-stage renal disease — yr</td>
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<td>54±36</td>
<td>51±27</td>
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<td>27 (100)</td>
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<td>18 (24)</td>
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<td>10 (100)</td>
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<td>12 (44)</td>
<td>10 (100)</td>
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<td>Azathioprine — no. (%)</td>
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<td>5 (19)</td>
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<td>Drug levels 12 mo after transplantation — ng/ml</td>
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<tr>
<td>Tacrolimus trough</td>
<td>10.1±6.0</td>
<td>10.3±5.9</td>
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<tr>
<td>Cyclosporine peak</td>
<td>647±342</td>
<td>643±365</td>
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<tr>
<td>Sirolimus</td>
<td>17±2</td>
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<td>18±9</td>
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<tr>
<td>Lupus anticoagulant — no. (%)</td>
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<td>27 (100)</td>
<td>10 (100)</td>
</tr>
<tr>
<td>Anti-β₂-glycoprotein I antibodies — no. (%)</td>
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<td>5 (19)</td>
<td>2 (20)</td>
</tr>
<tr>
<td>Anticardiolipin antibodies — no. (%)</td>
<td>0</td>
<td>7 (26)</td>
<td>4 (40)</td>
</tr>
</tbody>
</table>

* Plus–minus values are means ±SD. APS denotes antiphospholipid syndrome, GFR glomerular filtration rate, NA not applicable, and SLE systemic lupus erythematosus.
used. Allograft survival rates were censored at the time of death; rates were calculated with the use of the Kaplan–Meier method and analyzed with the log-rank test. For details of the statistical analysis, see the Supplementary Appendix.

RESULTS

ANTIPHOSPHOLIPID SYNDROME NEPHROPATHY

Activation of the mTORC Pathway in Endothelial Cells

To investigate the activation state of the mTORC pathway in the renal vessels of patients with primary antiphospholipid syndrome nephropathy, we evaluated the phosphorylation of S6 ribosomal protein (S6RP) and AKT (phosphorylation at Ser473), which reflect the activation of mTORC1 and mTORC2, respectively. Although immunohistochemical analysis combined with careful morphologic analysis confirmed that cells in which S6RP or AKT (Ser473) was phosphorylated had morphologic features of vascular endothelial cells (Fig. S1C in the Supplementary Appendix). Neither S6RP nor AKT (Ser473) was activated in vascular endothelial cells that expressed CD105 and CD31, were not activated in cells expressing alpha-smooth-muscle actin (Fig. 1A, 1B, and 1C, and Fig. S1B in the Supplementary Appendix). Double immunostaining revealed very few vascular sections that were positive for phosphorylated S6RP or phosphorylated AKT (Ser473) in controls, many vessels were positive in sections from patients with antiphospholipid syndrome nephropathy (Fig. 1). Most positive cells were located in prominent vascular lesions, and mTORC1 and mTORC2 activation occurred in the same vessels (Fig. S1A in the Supplementary Appendix). Although double immunostaining experiments showed that S6RP and AKT (Ser473) was activated in vascular endothelial cells that expressed CD105 and CD31, they were not activated in cells expressing alpha-smooth-muscle actin (Fig. 1A, 1B, and 1C, and Fig. S1B in the Supplementary Appendix).

To determine whether antiphospholipid antibodies directly activate the mTORC pathway, we incubated HMEC-1, a human microvascular endothelial-cell line, with either normal human IgG antibodies or polyclonal antiphospholipid antibodies isolated from patients with the antiphospholipid syndrome who had a clinical history of vascular injury. The purity of the IgG antibodies was assessed with the use of sodium dodecyl sulfate–polyacrylamide-gel electrophoresis (Fig. S3 in the Supplementary Appendix). The polyclonal antiphospholipid antibodies from the patients induced a marked increase in S6RP and AKT (Ser473) phosphorylation, whereas the normal human IgG antibodies did not (Fig. 2). There was also a significant correlation between the phosphorylation of AKT (Ser473) and the titers of anticardiolipin antibodies ($R^2 = 0.47$, $P = 0.01$) and anti–β$_2$-glycoprotein I antibodies ($R^2 = 0.36$, $P = 0.04$). We confirmed these results in primary cultures of cells from human umbilical veins (Fig. S4 in the Supplementary Appendix).

Because anti-HLA antibodies, particularly anti-HLA class I antibodies, may activate the mTORC pathway in endothelial cells during humoral rejection, we performed HLA typing of the human microvascular endothelial cell line (HLA-A01, A68, B35, B58, DR18, and DR12) and searched for specific anti-HLA antibodies. Antibodies directed against the HLA haplotypes identified in the human microvascular endothelial cell line might contribute to lesion development. Very few cells were positive for proliferating-cell nuclear antigen (PCNA) in renal vessels from controls or patients with lupus nephritis in the absence of the antiphospholipid syndrome. However, there was an increase in the number of PCNA-positive cells in the renal vasculature of patients with primary or secondary antiphospholipid syndrome (Fig. S2A and S2C in the Supplementary Appendix). Double immunostaining with antibodies against Ki-67, a protein that is selectively expressed in proliferating cells, and with antibodies to alpha–smooth-muscle actin revealed proliferation of both endothelial cells and vascular smooth-muscle cells (Fig. S2B and S2C in the Supplementary Appendix), suggesting the possibility of cross-talk between the two cellular compartments, which has been reported in other conditions (e.g., mechanical endothelial injury, antibody-mediated rejection, and shear stress). 

ANTIPHOSPHOLIPID ANTIBODIES AND mTORC PATHWAY ACTIVATION

Activation of the mTORC Pathway and Vascular Proliferation

The activation of the mTORC pathway can trigger cell proliferation. Therefore, there is intimal hypercellularity in antiphospholipid syndrome nephropathy, we posited that an increase in cell proliferation...
were detected in only 6 of 26 patients and controls from whom IgG antibodies were isolated (Table S2 in the Supplementary Appendix). However, these antibodies were found in both healthy volunteers and patients with the antiphospholipid syndrome, indicating that anti-HLA antibodies did not account for mTORC activation in microvascular endothelial cells. Phosphorylation of AKT (Ser473) and S6RP was completely abolished when the cells were treated with PP242, a selective mTOR kinase inhibitor (Fig. S5 in the Supplementary Appendix). Pretreatment with LY294002, an inhibitor of PI3K, also completely prevented AKT (Ser473) activation, indicating that the recruitment of AKT to the cell membrane is necessary for antiphospholipid-antibody–induced mTORC signaling (Fig. S5 in the Supplementary Appendix). Consistent with this finding was the observation that antiphospholipid antibodies stimulated the phosphorylation of AKT on Thr308, the target residue of the PI3K pathway (Fig. 2).

**Figure 1.** Activation of the mTORC Pathway in Patients with the Antiphospholipid Syndrome and in Patients with Systemic Lupus Erythematosus with or without the Antiphospholipid Syndrome.

Panel A shows the results of immunostaining of phosphorylated AKT (Ser473) (green) and the endothelial-cell marker CD105 (red) in kidney-biopsy specimens from four groups of patients: controls, patients who had primary antiphospholipid syndrome (APS) nephropathy, patients who had systemic lupus erythematosus (SLE) in the absence of APS, and patients who had SLE and APS. Panel B shows the results of double immunostaining of phosphorylated S6 ribosomal protein (S6RP) (red) and alpha–smooth-muscle actin (α-SMA) (green) in kidney specimens from the four groups of patients. Scale bars in Panels A and B represent 10 μm. Panel C shows the percentage of vascular kidney specimens that were positive for phosphorylated AKT (Ser473) and phosphorylated S6RP in relation to the total number of vascular specimens. Data are means ±SE. Analysis of variance was performed, followed by the Tukey–Kramer test. P values were calculated for patients with APS versus controls and for patients who had SLE and APS versus patients who had SLE without APS.
We wondered whether these observations applied to patients with antiphospholipid syndrome nephropathy. In human microvascular endothelial cells 1 hour of exposure to sirolimus completely inhibited the phosphorylation of S6RP induced by antiphospholipid antibodies but failed to prevent the phosphorylation of AKT (Ser473) (Fig. S5 in the Supplementary Appendix). However, when cells treated with antiphospholipid antibodies were exposed to sirolimus for 48 hours, phosphorylation of both S6RP and AKT (Ser473) was inhibited, and the cells appeared to be viable and looked normal (Fig. S5 in the Supplementary Appendix). These results are consistent with previous observations that prolonged exposure to sirolimus can lead to mTORC2 inhibition.24-26

**EFFECTS OF SIROLIMUS**

Among the 37 kidney-transplant recipients with antiphospholipid antibodies, 10 were receiving sirolimus as an immunosuppressive drug. We compared this group of patients with a matched control group of 74 transplant recipients in whom antiphospholipid antibodies were not detected; 56 of these patients were receiving a calcineurin inhibitor and 18 were receiving sirolimus. Biopsies performed at 3 and 12 months after transplantation revealed markedly increased phosphorylation of S6RP and AKT (Ser473) in vascular endothelial cells from the transplant recipients with antiphospholipid antibodies who were not receiving sirolimus as compared with cells from the recipients without antiphospholipid antibodies (Fig. 3A and 3B, and Fig. S6 in the Supplementary Appendix). As expected, the number of vascular sections with positive test results for phosphorylated S6RP and AKT (Ser473) was dramatically reduced among the patients with antiphospholipid antibodies who were treated with sirolimus (Fig. 3A and 3B, and Fig. S6 in the Supplementary Appendix). No hyperplasia of either endothelial cells or vascular smooth-muscle cells was detected in renal specimens from transplant recipients with antiphospholipid antibodies who were receiving sirolimus (Fig. 3A and 3B, and Fig. S6 in the Supplementary Appendix). During the first year after transplantation, very few renal lesions developed in these patients as compared with transplant recipients with the antiphospholipid syndrome who were not receiving sirolimus (Fig. 3C, and Fig. S8 in the Supplementary Appendix), despite the fact that the findings on examination of specimens from pretransplantation biopsies were similar among the three groups (i.e., the patients with antiphospholipid antibodies who were receiving sirolimus, the patients with antiphospholipid antibodies who were not receiving sirolimus, and the patients without antiphospholipid antibodies) (data not shown).

Consistent with the reduced number of renal lesions in patients with antiphospholipid antibodies who were receiving sirolimus was the observation that sirolimus treatment appeared to protect the kidney transplants from loss of function. At 12 months after transplantation, the GFR, as measured by the clearance of iohexol, was significantly reduced in transplant recipients with antiphospholipid antibodies who were not receiving sirolimus but was stable in transplant recipients with antiphospholipid antibodies who were receiving sirolimus. The GFR in this group was similar to that in the transplant recipients without antiphospholipid antibodies (Fig. 3D).
At a mean follow-up of 82±35 months, a Kaplan-Meier survival analysis showed a clear improvement in allograft survival among patients with antiphospholipid antibodies who were receiving sirolimus as compared with patients with antiphospholipid antibodies who were not receiving sirolimus (Fig. 3E). In fact, whereas only 3 of the 27 patients with antiphospholipid antibodies who did not receive sirolimus still had a functional kidney 144 months after transplantation (11%), 7 of the 10 patients with antiphospholipid antibodies who were treated with sirolimus still had well-preserved kidney function at that time (70%). However, sirolimus administration did not enhance allograft survival in transplant recipients who did not have antiphospholipid antibodies (Fig. 3E). An analysis of clinical variables known to affect graft outcome, such as cold ischemia time (the time elapsed between procurement of the organ and transplantation) and immunologic variables (e.g., inflammation of the microcirculation and C4d deposition in renal capillaries), showed no significant association with prolonged kidney survival in transplant recipients with antiphospholipid antibodies who received sirolimus (Table 1, and Table S3 in the Supplementary Appendix). Similarly, among the patients with antiphospholipid antibodies, the levels and types of these antibodies did not differ significantly, regardless of the immunosuppressive regimen (Table S4 in the Supplementary Appendix). All transplant recipients with antiphospholipid antibodies received anticoagulant medications, and no thrombotic lesions were detected in the damaged vessels of these patients, regardless of whether they received sirolimus. Levels of calcineurin inhibitor in blood were similar among transplant recipients with and those without antiphospholipid antibodies who were not receiving sirolimus. This argues against a possible role for this drug in mTORC pathway activation, as has been reported in cancer cells.27

**ACTIVATION OF mTORC PATHWAY IN CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME**

To investigate whether sirolimus could be beneficial to organs other than the kidney that are damaged in the antiphospholipid syndrome, we assessed the vascular changes and the state of mTORC pathway activation in patients with catastrophic antiphospholipid syndrome who had died (Table S5 in the Supplementary Appendix). In these patients, we noted the same marked neointimal formation associated with severe constriction of the vessel lumens in both the carotid and left anterior descending arteries (Fig. S9 in the Supplementary Appendix). Immunohistochemical analysis showed a marked increase in the phosphorylation of S6RP and AKT (Ser473) in the endothelial cells of damaged vessels (Fig. S9 in the Supplementary Appendix). Few of the neointimal cells that displayed the typical morphologic features of infiltrating inflammatory cells were also positive for markers of mTORC pathway activation.

**DISCUSSION**

The molecular pathways that lead to the intimal hyperplasia that accompanies the most severe forms of the antiphospholipid syndrome are unknown. The fact that such lesions develop, despite adequate anticoagulation, suggests that the coagulation defect itself is not central to the lesions. Our findings, based on a combination of clinical studies and in vitro experiments, indicate that the mTORC pathway plays a crucial role in the development of vascular lesions associated with the antiphospholipid syndrome. The development of intimal hyperplasia was associated with the activation of both mTORC1 and mTORC2, and the greatest activation was observed in vessels with the most prominent lesions. Among kidney-transplant recipients with the antiphospholipid syndrome who were treated with sirolimus, mTORC pathway activation was averted, preventing the development of vascular lesions. Anti-HLA antibodies were also associated with neointimal hyperplasia, the proliferation of vascular smooth-muscle cells, and mTORC pathway activation.21,28 However, our data argue against a role for these antibodies in the vascular phenotype observed in our patients. Hence, mTORC pathway activation in endothelial cells appears to be a more general mechanism of antibody-mediated vascular injury.

The mechanisms by which antiphospholipid antibodies lead to cell activation are incompletely elucidated. It has been shown that antiphospholipid antibodies first associate with β₂-glycoprotein 1 antigens29 and then bind to membrane receptors that mediate extracellular signaling, ultimately activating endothelial cells and platelets.30,31 In the context of thrombosis,
A Phosphorylated ATK (Ser473) and CD105

B Phosphorylated S6RP and α-SMA

C Lesion Development

D GFR and Graft Survival
p38 mitogen-activated protein kinase and nuclear factor κB have been shown to be critical intermediates.\textsuperscript{32} Our data indicate that a different signaling pathway is involved in the development of intimal hyperplasia and suggest that mTORC plays a crucial role. We observed that antiphospholipid antibodies activated AKT (Ser473) at the plasma membrane in a PI3K-dependent manner. Some of the receptors activated by the antiphospholipid antibody-β\textsubscript{2}-glycoprotein I complexes trigger PI3K signaling.\textsuperscript{33} In this study, all the serum specimens that we examined from patients with the antiphospholipid syndrome contained a mixture of anti-β\textsubscript{2}-glycoprotein I, cardiolipin, and anti-lupus anticoagulant antibodies that variably activated the endothelial mTORC pathway. Hence, it is tempting to speculate that the activation of distinct signaling pathways drives the response of endothelial cells toward proliferation or thrombosis.

The prevention of thrombosis is a major therapeutic goal in patients with the antiphospholipid syndrome. However, despite adequate anticoagulation, renal lesions develop in some patients\textsuperscript{6,7} and recur after transplantation, often leading to graft loss.\textsuperscript{9} Our data suggest that inhibition of the mTORC pathway in kidney-transplant recipients who have antiphospholipid antibodies protects the transplanted graft and, more important, prevents graft loss by preventing the development of intimal hyperplasia. We observed that the levels of antiphospholipid antibodies and the immunologic variables known to affect graft survival were similar in the transplant recipients, irrespective of whether they were receiving sirolimus, an observation that rules out a specific effect of sirolimus on the immune system, as previously reported in some immunologic disorders.\textsuperscript{34,35} In addition, we found that the beneficial effect of sirolimus was independent of coagulation, since all the transplant recipients with antiphospholipid antibodies had adequate anticoagulation. Antiphospholipid antibodies elicited mTORC signaling in vitro in the absence of a functional coagulation cascade. On the other hand, although we cannot formally exclude the possibility that sirolimus has a beneficial secondary effect on endothelial cells, the observation that in other disorders sirolimus administration prevents neointimal formation by modulating endothelial cells supports the idea that sirolimus acts directly on endothelial cells.\textsuperscript{13,14,36} The involvement of mTORC in the pathogenesis of tubular and glomerular damage has been shown previously.\textsuperscript{12,26,37,38} The fact that mTORC was predominantly activated in vessels from patients with antiphospholipid syndrome nephropathy suggests a pleiotropic effect of mTORC function in renal pathophysiology. Our study suggests that a specific molecular pathway, the mTORC pathway, leads to intimal hyperplasia, which accompanies the most severe variants of the antiphospholipid syndrome.

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