Tacrolimus-Induced Hypertension
What’s Endothelial and Hematopoietic FKBP12 Got to Do With It?

Sean P. Didion

See related article, pp 1167–1175

Hypertension associated with solid-organ transplantation is of major clinical importance, because the degree of posttransplant hypertension has been shown to correlate with reductions in graft viability and survival.1–3 Effective blood pressure control with antihypertensives has been shown to enhance graft survival and reduce the risk of future cardiovascular disease and events in transplant recipients.3 The etiology of posttransplant hypertension is varied; however, hypertension produced by immunosuppressant therapy is an important cause of posttransplant-related hypertension. Tacrolimus (FK506) and cyclosporine A are 2 common immunosuppressants that have been shown to affect blood pressure. Thus, elucidation of mechanisms that contribute to posttransplant hypertension has major clinical implications.

In general, the type of immune response evoked by a particular set of antigens, such as that associated with organ transplantation, depends on the expansion of specific T-lymphocyte (T-cell) subsets, as well as the duration of antigen exposure, which, in the case of organ transplantation, is life long. Tacrolimus and cyclosporine A limit the immune response via inhibition of calcineurin activity, through similar but slightly different mechanisms. With tacrolimus, the mechanism of calcineurin inhibition involves tacrolimus binding to its cytosolic binding partner, the ubiquitously expressed immunophilin FKBP12. Tacrolimus/FKBP12, along with calcium and calmodulin, forms a complex with calcineurin, thereby inhibiting calcineurin’s phosphatase activity. Calcineurin signaling has been shown to regulate expression of a number of cytokines, including interleukin (IL)-2, IL-3, IL-4, and interferon-γ, as well as expression of the IL-2 receptor, which have been implicated in T-cell activation.4 For example, reducing IL-2 signaling with tacrolimus is important in limiting the immune response, because IL-2 promotes the expansion of cytotoxic T cells and has been shown to play an important role in immunologic memory.

In this issue of Hypertension, Chiasson et al5 report studies on the molecular mechanisms that contribute to hypertension produced by tacrolimus. Their goal was to test the hypothesis that tacrolimus can influence polarization of specific T-cell subsets that then contribute to the hypertension and endothelial dysfunction observed in humans and experimental models.

In the first half of their study, Chiasson et al5 treated wild-type (C57Bl/6) mice with tacrolimus (1 or 10 mg/kg per day IP) for 7 days. On day 7, blood pressure was significantly higher in mice that received tacrolimus, and the response appeared to be concentration dependent, with the highest concentration of tacrolimus (10 mg/kg per day) producing a 40- to 50-mm Hg rise in systolic pressure as compared with day 0. The degree of hypertension produced in this study is striking in terms of its magnitude. To put this in a frame of reference, this is very similar to the amount of hypertension attained with angiotensin II infusion in mice with the commonly used high pressor dose of 1000 ng/kg per minute.6 Nonetheless, the amount of hypertension produced with tacrolimus in the present study is consistent with previous studies.2

In addition to increasing blood pressure, tacrolimus produced impairment of endothelium-dependent, but not -independent, relaxation in thoracic aorta (ex vivo). The degree of endothelial dysfunction produced by the highest concentration of tacrolimus was associated with near complete inhibition of endothelial function. Because relaxation in mouse aorta is mediated predominately by endothelial NO synthase (eNOS),8 these data suggest that the impairment of endothelial function produced by tacrolimus most likely reflects a reduction in eNOS expression and/or activity. These findings are supported, in part, by previous studies implicating alterations in eNOS phosphorylation, as well as increased endothelin expression with tacrolimus.7 Taken together, the evidence clearly demonstrates that tacrolimus can have dramatic effects on blood pressure and endothelial function.

To test the hypothesis that the increase in blood pressure and endothelial dysfunction produced by tacrolimus reflect changes in specific T-cell subsets, the authors examined total T-helper cell (CD3+/CD4+; Thelper), regulatory T cell (CD4+/FoxP3+; Treg), and Th17 cell (CD4+/IL-17a+; a more recently defined Thelper cell population) numbers in spleens isolated from vehicle- and tacrolimus-treated mice as assessed using flow cytometry. Tacrolimus was associated with a lower numbers of Thelper cells expressed as a percentage of total splenocytes. Perhaps most important, tacrolimus was associated with lower numbers of anti-inflammatory Treg cells while producing an increase in the number of proinflammatory Th17 cells. These data suggest that the increases in blood pressure and reductions in endothelial function pro-
Tacrolimus or Endothelial and Hematopoietic FKBP12 Deficiency (FKBP12EC)

\[ T_{\text{reg}} \downarrow \quad T_{\text{H17}} \]

Inflammatory Phenotype (i.e., increased IL-6, IL-17a, IL-23, and IL-23 levels)

Hypertension and Endothelial Dysfunction

Figure. The impact of tacrolimus or endothelial and hematopoietic deletion of FKBP12 (FKBP12EC mice) on blood pressure and endothelial function. Both pharmacological and genetic evidence from the present study by Chiasson et al.\(^\text{a}\) provide strong evidence supporting a role for FKBP12 in driving an inflammatory phenotype that most likely contributes to the hypertension and endothelial dysfunction observed in humans and experimental models with tacrolimus treatment. The inflammatory phenotype characterized by inhibition or deletion of FKBP12 involves a shift in the balance of proinflammatory T-helper 17 (T_{H17}) and anti-inflammatory regulatory T cell (T_{reg}) cell numbers, as well as increases in levels of proinflammatory cytokines.

Reduced with tacrolimus are associated with a shift in the balance of proinflammatory versus anti-inflammatory T-cell subsets (Figure).

Although these findings are important, one should take into consideration some of the potential limitations common to pharmacological approaches, such as that used in the present study. First, the use of tacrolimus at 1 and 10 mg/kg per day as in the present study is higher compared with that used previously in the literature in experimental animals, raising some concern that the effects may be related to nonspecific effects unrelated to calcineurin inhibition. In addition, it is important to point out that common clinical doses are also much lower than those used experimentally. Second, because the authors did not measure steady-state levels of tacrolimus in plasma from tacrolimus-treated animals, direct comparisons with clinical doses of tacrolimus are difficult. Comparisons are also complicated by the fact that there may be species differences and/or dosing differences, for example, clinically it is well known that higher dosing of tacrolimus is required in pediatric transplant patients than adults.

However, it is in the second half of the present study that the authors were able to circumvent any potential limitations associated with pharmacology by directly addressing the role of the tacrolimus binding partner FKBP12 using a genetic approach that is both innovative and elegant in its design. The authors took advantage of the fact that, when Fkbp12-deficient mice were created, their design included loxP sites. Inclusion of loxP sites allows one to exploit such sites in combination with Cre recombinase (Cre), a technique referred to as Cre-Lox recombination. This is exactly the approach the authors used when they bred Fkbp12 (lox) mice with transgenic mice that express Cre driven by a Tie2 promoter. Thus, Fkbp12 expression was selectively eliminated from endothelial and hematopoietic cells (FKBP12EC-deficient mice), allowing the authors to examine the effect of Fkbp12 deletion in these 2 cell types.

The authors found that FKBP12EC mice were associated with a significant degree of hypertension (blood pressure was \( \approx 30 \) mm Hg higher in FKBP12EC mice versus controls). In addition, FKBP12EC mice displayed marked impairment of endothelial function. These findings are very similar to that observed in tacrolimus-treated mice in the first half of the study. The authors attempted to link the alterations in endothelial function (and perhaps blood pressure) with alterations in eNOS expression, because they found that total eNOS was reduced in aorta of FKBP12EC mice. Although the aorta is a large conduit vessel, expression of eNOS at this level does not always correlate with alterations at the level of resistance vessels. Thus, it would have been interesting if the authors would have also examined vascular responses at the level of the microcirculation. Such studies would have been very interesting and would have helped to provide greater insight into whether the hypertension in the FKBP12EC mice is because of vascular alterations at the microvascular level. In addition, measures of relative eNOS expression do not always equate with eNOS activity; thus, it would have been interesting to also measure eNOS phosphorylation and activity in FKBP12EC mice and correlate that with T-cell number and inflammatory cytokine levels.

With regard to T-cell numbers in FKBP12EC mice, the authors found that Th17 cell numbers were higher and Treg cell numbers were lower, consistent with their findings in tacrolimus-treated mice. Somewhat surprisingly, considering that tacrolimus was associated with reduction in anti-inflammatory T_{reg} cells, there were no alterations in plasma levels of anti-inflammatory cytokines with FKBP12 deficiency. In contrast, higher plasma levels of the proinflammatory cytokines, IL-6, IL-17, IL-21, and IL-23, characterized FKBP12EC-deficient mice. Interestingly, IL-6 and IL-17 have been shown to contribute to endothelial dysfunction and hypertension produced by angiotensin II.\(^9\)

It is tempting to speculate that IL-6 may explain the observed reduction in eNOS expression in FKBP12EC mice, because IL-6 has been shown to inhibit eNOS expression via a signal transducer and activator of transcription-3 (STAT3)-dependent mechanism.\(^10\) Although the authors did not measure STAT3 phosphorylation in vascular tissue, they did observe higher levels of STAT3 phosphorylation in splenocytes of FKBP12EC mice. Thus, in future studies, it will be relevant to determine whether these same cytokines contribute directly to the hypertension and endothelial dysfunction in tacrolimus-treated mice, as well as FKBP12EC mice.

Several additional questions arise from the present study. For example, what happens to cytotoxic T-cell numbers with endothelial and hematopoietic cell Fkbp12 deficiency? Is the endothelial dysfunction in FKBP12EC mice associated with higher levels of infiltrating immune cells, such as macrophages? Can tacrolimus treatment produce any additional increases in blood pressure in FKBP12EC mice? If no further
increase in pressure is observed with tacrolimus, such data would provide further proof that it is indeed expression of FKBP12 in endothelial and hematopoietic cells that is responsible for the observed hypertension. Clinically, will it be possible to suppress the effects of tacrolimus on Th17 cell polarization to limit the hypertension associated with this drug and improve patient outcomes? As it stands, the findings of the present study, using both pharmacological and genetic approaches, strongly support a regulatory role of FKBP12 in directing T-cell proliferation in a manner that favors expansion of Th17 cells and a reduction in Treg cell number, thereby providing a distinct molecular mechanism that explains the hypertension produced by tacrolimus.

Sources of Funding
S.P.D. is supported by the National Institutes of Health, National Heart, Lung, and Blood Institute (HL089884 and HL107632).

Disclosures
None.

References
Tacrolimus-Induced Hypertension: What's Endothelial and Hematopoietic FKBP12 Got to Do With It?
Sean P. Didion

Hypertension. 2011;57:1058-1060; originally published online April 25, 2011;
doi: 10.1161/HYPERTENSIONAHA.111.172320

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/57/6/1058

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/