The Role of TWEAK in Calcineurin Inhibitor Nephrotoxicity (CNT)

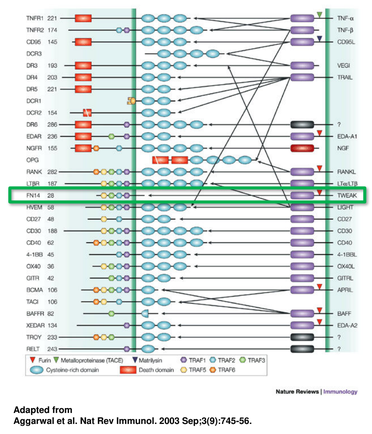
Meike Claus[1,3], Rana Herro[1], Dennis Wolf[2], Konrad Buscher[2], Stefan Rudloff[3], Uyen Huynh-Do[3], Linda Burkly[4], Michael Croft[1] and Daniel Sidler[1,3],

Division of Immune Regulation, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037, USA.[1]
Division of Cell Biology, La Jolla Institute for Allergy and Immunology, La Jolla, CA 92037, USA.[2]
University Hospital Insel, Bern, Department for Nephrology and Hypertension, 3010 Bern, Switzerland. [3]
Department of Immunology, Biogen Idec, 14 Cambridge Center, Cambridge, MA 02142, USA.[4]

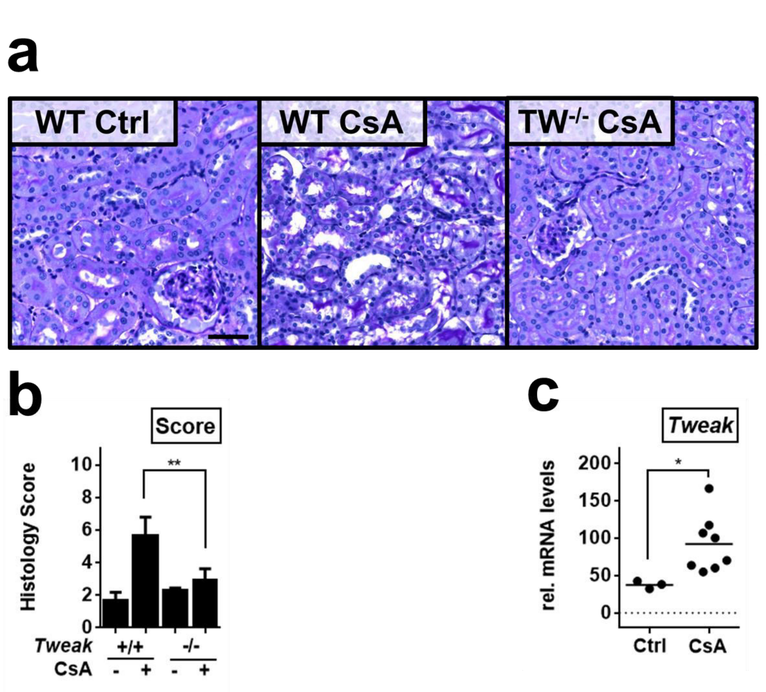
ABSTRACT

Calcineurin inhibitor toxicity (CNT) is a frequent disease entity of transplanted renal grafts and autochthone kidneys in patients under the long-term prescription with Calcineurin inhibitors, notably Cyclosporin A (CsA) and Tacrolimus. Here, we show an indispensable role of the TNF superfamily molecule TWEAK (TNFSF12) in the pathogenesis of acute CNT lesions in mice. A deficiency in TWEAK resulted in limited tubulotoxicity after CsA exposure, which correlated with diminished expression of inflammatory cytokines and reduced intraparenchymal infiltration with immune cells. Furthermore, combined treatment of recombinant TWEAK (rTWEAK) and CsA induced synergistic nephrotoxicity, while respective monotherapies elicited only moderate disease. We further identified tubular epithelial cells of the kidney as major targets of CsA's activity and describe TWEAK's receptor Fn14 (TNFRSF12A) as a highly CsA-inducible gene. Intriguingly, CsA pretreatment sensitized tubular epithelial cells specifically to the pro-inflammatory activities of rTWEAK in vitro. These findings support the importance of tubular epithelial cells as cellular targets of CsA's toxicity and introduce TWEAK as a critical contributor to CNT pathogenesis.

The TWEAK/Fn14 pathway in chronic inflammation and fibrosis



TWEAK deficiency protects from acute CNT *in vivo*



CsA rapidly induces Fn14 in tubular epithelial cells *in vivo* and *in vitro*

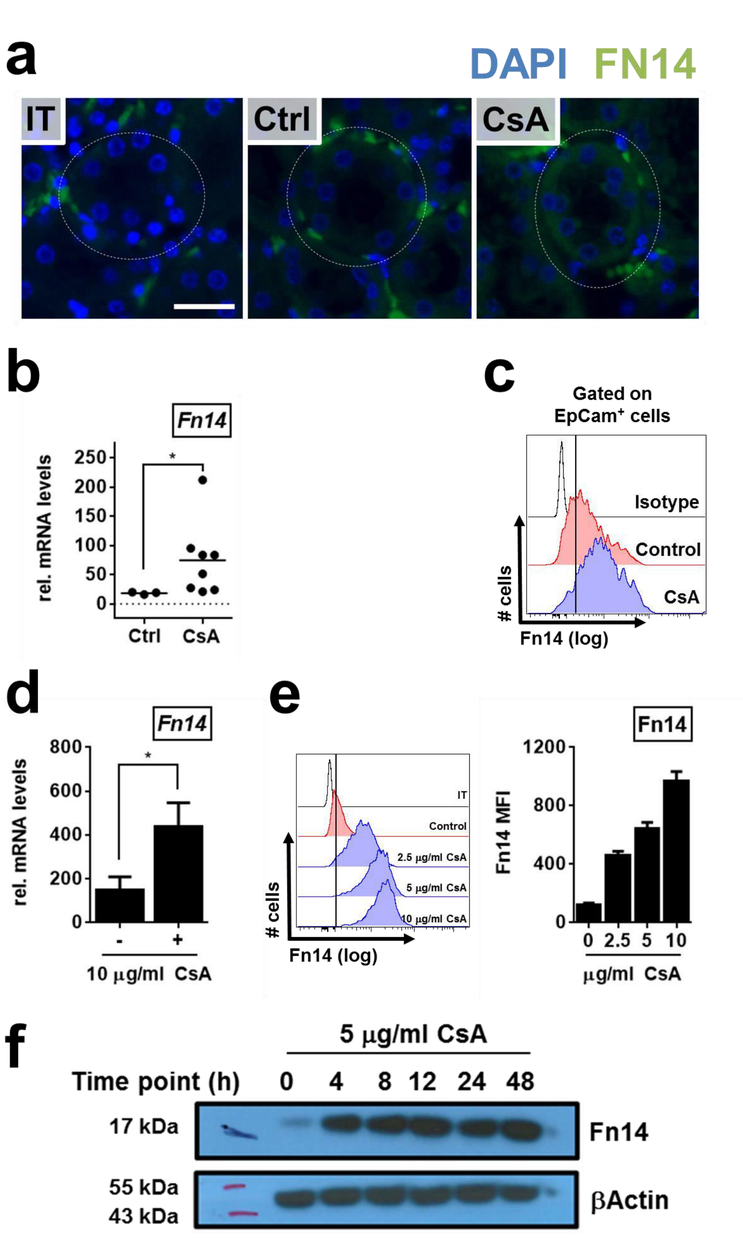
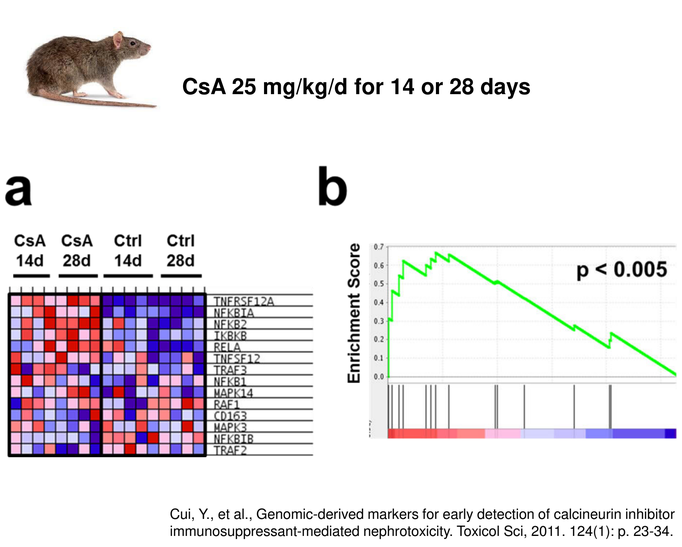


Figure 2: TWEAK deficiency protects acute CNT. a) PAS stainings from juxtamedullary regions of WT (Wild type) and *Tweak* deficient ($TW^{-/-}$) animals treated with Vehicle (Ctrl) or 100 mg/kg/d CsA for two days. Bar represents 50 µm. **b**) Semi quantitative disease severity based on a histological score composed of tubular epithelial swelling, loss of brush border, vacuolar degeneration, necrotic tubules and cast formation (score 0-4, maximal score 20). **c**) Expression of *Tweak* transcripts in the kidneys at the end of the experiment. **d)** Infiltration of $Ly6G^{hi}$ neutrophilic granulocytes and $Ly6c^{hi}$ inflammatory monocytes in the various experimental groups. **e)** Expression of Ccl5 transcripts in the kidney at the end of the experiment. Each data point represents one animal.

Figure 3. CsA induces Fn14 in tubular epithelial cells in vivo and in vitro. a) Fn14 expression in the kidney after with 100 mg/kg/d CsA for two days. **b)** Expression of Fn14 transcripts in the kidneys on day 3 in WT animals. **c)** Expression of Fn14 on EpCam-positive kidney cells from animals treated with Vehicle (Control) or 100 mg/kg/d CsA for two days. Vertical line indicates MFI of Control animal. **d-f)** mRNA and protein Fn14 expression in MCT cells treated with Control or indicated concentrations of CsA for 48 hours.

The TWEAK/Fn14 pathway is enriched in chronic CNT lesions in rats *in vivo*



Cui, Y., et al., Genomic-derived markers for early detection of calcineurin inhibitor immunosuppressant-mediated nephrotoxicity. Toxicol Sci, 2011. 124(1): p. 23-34.

Figure 1: The TWEAK/Fn14 pathway is highly enriched in chronic CNT lesions. a-b) Gene set enrichment analysis of kidney specimen from rats treated with Control or 25 mg/kg/d CsA for 14 or 28 days. Fn14 (TNFRSF12A) and intracellular signaling and effector molecules are significantly enriched among the induced transcripts in CsA-exposed animals when compared to control treated animals (FDR < 0.02 for Ctrl [d14+d28] vs. CsA [d14+d28], FDR < 0.05 for individual time points).

CsA sensitizes tubular epithelial cells to TWEAK's inflammatory activity

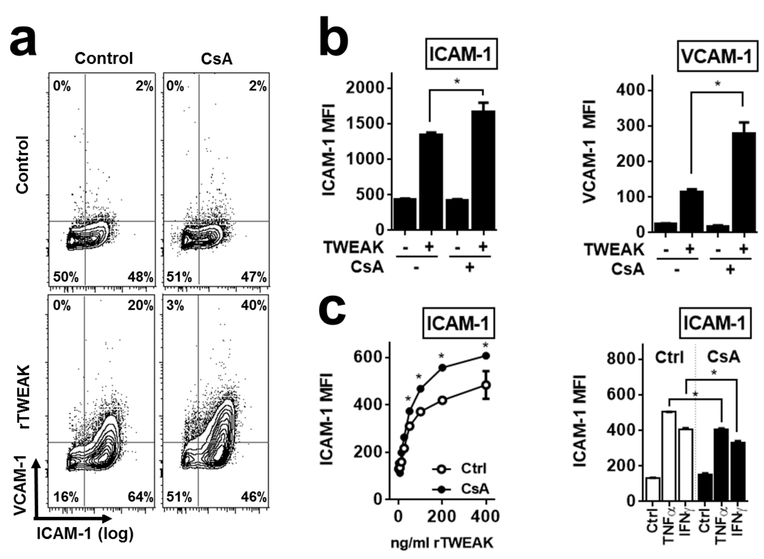


Figure 4: **CsA sensitizes cells specifically to TWEAK's inflammatory activity**. **a-c)** ICAM-1 and VCAM-1 expression on MCT cells treated with 10 ug/ml CsA for 24 hours, followed by IgG or 100 ng/ml TWEAK-Fc, 25 ng/ml TNFα or 25 ng/ml IFNγ for additional 24 hours.

CsA and TWEAK synergize in their nephrotoxic potential in *vivo*

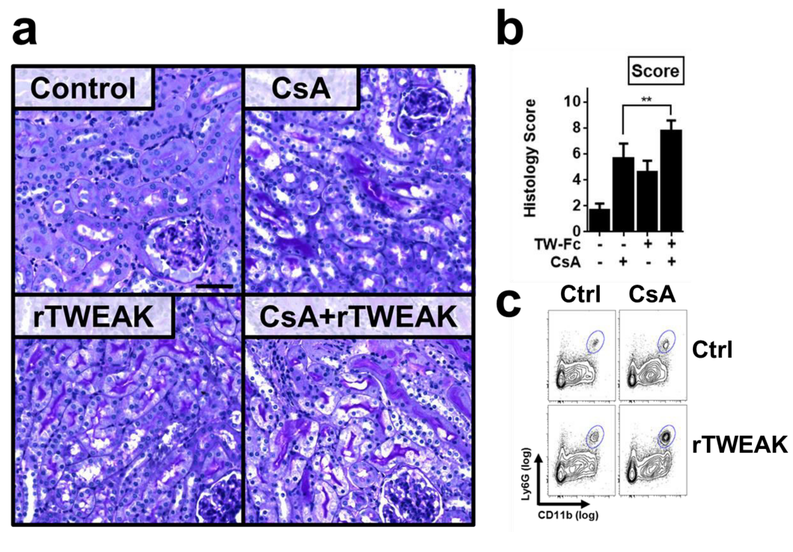


Figure 5: TWEAK and CsA synergize to induce acute tubulotoxicity in vivo. a) PAS stainings from juxtamedullary regions from WT (Wildtype) animals treated with Control, 100 mg/kg/d CsA, 75 ug/d rTWEAK or the combination thereof for two consecutive days. **b)** Semi quantitative disease severity of the various experimental **c)** Intrarenal infiltration of Ly6Ghi neutrophils

FNSNF Fonds National Suisse Schweizerischer Nationalfonds Fondo Nazionale Svizzero Swiss National Science Foundation
Biogen
Marie Curie Co-funding IKPP