



Acknowledgments

Our thanks to Benjamin L. Viglianti, Andrew N. Fontanella, and Kelly Kennedy for their thoughtful discussions regarding this commentary. We also thank Chamali Wickramasekara for technical help and the creativity of Isabel Cardenas-Navia. This work was supported by NIH/NCI grants CA40355 and CA42745.

Address correspondence to: Mark W. Dewhirst, Duke University Medical Center, Box 3455, Room 201 MSRB Research Drive, Durham, North Carolina, USA. Phone: (919) 684-4180; Fax: (919) 684-8718; E-mail: dewhi001@mc.duke.edu.

- Folkman, J. 1974. Tumor angiogenesis. *Adv. Cancer Res.* **19**:331–358.
- Secomb, T.W., Hsu, R., Dewhirst, M.W., Klitzman, B., and Gross, J.F. 1993. Analysis of oxygen transport to tumor tissue by microvascular networks. *Int. J. Radiat. Oncol. Biol. Phys.* **25**:481–489.
- Nordmark, M., et al. 2005. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. *Radiother. Oncol.* **77**:18–24.
- Hockel, M., et al. 1996. Association between tumor hypoxia and malignant progression in advanced cancer of the uterine cervix. *Cancer Res.* **56**:4509–4515.
- Hockel, M., et al. 1998. Tumor hypoxia in pelvic recurrences of cervical cancer. *Int. J. Cancer.* **79**:365–369.
- Vaupel, P., and Harrison, L. 2004. Tumor hypoxia: causative factors, compensatory mechanisms, and cellular response. *Oncologist.* **9**(Suppl. 5):4–9.
- Matsumoto, S., et al. 2008. Low-field paramagnetic resonance imaging of tumor oxygenation and glycolytic activity in mice. *J. Clin. Invest.* **118**:1965–1973.
- Manzoor, A., Yuan, H., Palmer, G., Viglianti, B., and Dewhirst, M. 2008. Imaging hypoxia. In *Molecular imaging: principles and practice*. R. Weissleder, et al., editors. B.C. Decker. Hamilton, Ontario, Canada. In press.
- Padhani, A.R., Krohn, K.A., Lewis, J.S., and Alber, M. 2007. Imaging oxygenation of human tumours. *Eur. Radiol.* **17**:861–872.
- Lewis, J.S., and Welch, M.J. 2001. PET imaging of hypoxia. *QJ Nucl. Med.* **45**:183–188.
- Neeman, M., Dafni, H., Bukhari, O., Braun, R.D., and Dewhirst, M.W. 2001. In vivo BOLD contrast MRI mapping of subcutaneous vascular function and maturation: validation by intravital microscopy. *Magn. Reson. Med.* **45**:887–898.
- Cooper, R.A., et al. 2000. Tumour oxygenation levels correlate with dynamic contrast-enhanced magnetic resonance imaging parameters in carcinoma of the cervix. *Radiother. Oncol.* **57**:53–59.
- Lyng, H., et al. 2001. Assessment of tumor oxygenation in human cervical carcinoma by use of dynamic Gd-DTPA-enhanced MR imaging. *J. Magn. Reson. Imaging.* **14**:750–756.
- Zhao, D., Jiang, L., and Mason, R.P. 2004. Measuring changes in tumor oxygenation. *Methods Enzymol.* **386**:378–418.
- Gatenby, R.A., and Gillies, R.J. 2004. Why do cancers have high aerobic glycolysis? *Nat. Rev. Cancer.* **4**:891–899.
- Schroeder, T., et al. 2005. Spatial heterogeneity and oxygen dependence of glucose consumption in R3230Ac and fibrosarcomas of the Fischer 344 rat. *Cancer Res.* **65**:5163–5171.
- Zu, X.L., and Guppy, M. 2004. Cancer metabolism: facts, fantasy, and fiction. *Biochem. Biophys. Res. Commun.* **313**:459–465.
- Robey, I.F., Lien, A.D., Welsh, S.J., Baggett, B.K., and Gillies, R.J. 2005. Hypoxia-inducible factor-1alpha and the glycolytic phenotype in tumors. *Neoplasia.* **7**:324–330.
- Ling, C.C., et al. 2000. Towards multidimensional radiotherapy (MD-CRT): biological imaging and biological conformality. *Int. J. Radiat. Oncol. Biol. Phys.* **47**:551–560.
- Chao, K.S., et al. 2001. A novel approach to overcome hypoxic tumor resistance: Cu-ATSM-guided intensity-modulated radiation therapy. *Int. J. Radiat. Oncol. Biol. Phys.* **49**:1171–1182.
- Lee, N.Y., et al. 2008. Fluorine-18-labeled fluoromisonidazole positron emission and computed tomography-guided intensity-modulated radiotherapy for head and neck cancer: a feasibility study. *Int. J. Radiat. Oncol. Biol. Phys.* **70**:2–13.
- Alber, M., Paulsen, F., Eschmann, S.M., and Machulla, H.J. 2003. On biologically conformal boost dose optimization. *Phys. Med. Biol.* **48**:N31–N35.
- Malinen, E., Sovik, A., Hristov, D., Bruland, O.S., and Olsen, D.R. 2006. Adapting radiotherapy to hypoxic tumours. *Phys. Med. Biol.* **51**:4903–4921.
- Thorwarth, D., Eschmann, S.M., Paulsen, F., and Alber, M. 2007. Hypoxia dose painting by numbers: a planning study. *Int. J. Radiat. Oncol. Biol. Phys.* **68**:291–300.
- Sovik, A., et al. 2007. Radiotherapy adapted to spatial and temporal variability in tumor hypoxia. *Int. J. Radiat. Oncol. Biol. Phys.* **68**:1496–1504.
- Theysohn, J.M., et al. 2007. Subjective acceptance of 7 Tesla MRI for human imaging. *MAGMA*. doi:10.1007/s10334-007-0095-x.
- Kirsch, D.G., et al. 2007. A spatially and temporally restricted mouse model of soft tissue sarcoma. *Nat. Med.* **13**:992–997.

Linking adiponectin to proteinuria

Rexford S. Ahima

Department of Medicine, Division of Endocrinology, Diabetes, and Metabolism,
University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, USA.

Obesity predisposes toward renal disease independently of diabetes and hypertension. In this issue of the JCI, Sharma and colleagues assessed the role of adiponectin, an adipose-derived hormone, in the pathogenesis of albuminuria (see the related article beginning on page 1645). Obese African Americans had reduced adiponectin levels associated with albuminuria. Adiponectin deficiency in mice induced oxidative stress, fusion of podocyte foot processes in the kidney glomerulus, and urinary albumin excretion. Adiponectin treatment reversed these abnormalities, likely through activation of AMPK. The benefits of adiponectin were observed in diabetic and nondiabetic mice. These findings suggest that adiponectin is a biomarker for kidney disease and may be targeted for prevention and treatment.

Nonstandard abbreviations used: AdipoR, adiponectin receptor; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; HMW, high molecular weight; Nox, NADPH oxidase; ZO-1, zonula occludens-1.

Conflict of interest: R.S. Ahima has received research support from Biomeasure/Ipsen Inc. and served on scientific advisory boards of Biomeasure/Ipsen Inc. and Ethicon Endo-Surgery (Johnson & Johnson).

Citation for this article: *J. Clin. Invest.* **118**:1619–1622 (2008). doi:10.1172/JCI35655.

Obesity and kidney disease

The obesity epidemic has been linked to rising incidences of type 2 diabetes, cardiovascular disease, nonalcoholic fatty liver disease, sleep apnea, and cancer (1). Studies also indicate that obesity increases the risk of kidney disease independently of diabetes and hypertension (2, 3). Renal blood flow and glomerular filtration rate

are elevated in obesity and are related to increased levels of the protein albumin in the urine (albuminuria) (4). Epidemiological studies suggest that microalbuminuria, defined as a urine albumin/creatinine ratio of 30–300 μg/mg, increases cardiovascular morbidity (5). Furthermore, an albumin/creatinine ratio considered to be within the normal range (10–30 μg/mg) is associated with higher cardiovascular risk (6). Insulin resistance, oxidative stress, and inflammation have all been implicated in albuminuria and declining kidney function, but the underlying mechanisms are unclear (4).

Hypoadiponectinemia is related to albuminuria

In the current issue of the *JCI*, Sharma et al. describe a role of adiponectin in the pathogenesis of albuminuria (7). Adiponectin is

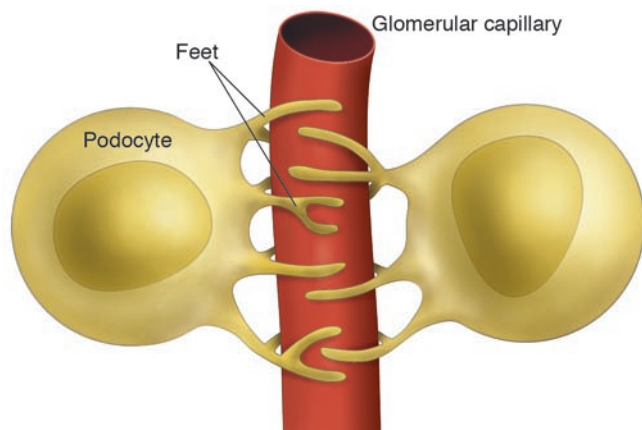


Figure 1
Schematic demonstrating the interdigitation of podocyte foot processes that surround glomerular capillaries.

secreted exclusively by adipocytes (8). The monomer is a 30-kDa protein with 3 distinct domains: N-terminal hypervariable region, collagenous stalk, and C-terminal globular domain (8, 9). Adiponectin circulates in plasma as various complexes: high-molecular weight (HMW; 12- to 36-mer), low-molecular weight (hexamer), and trimeric forms. Total and HMW adiponectin levels are more abundant in females and decline in obesity (8, 9). Low adiponectin levels are related to higher prevalence of type 2 diabetes, inflammation, and atherosclerosis, and these abnormalities are reversed by adiponectin treatment (8, 10). The insulin-sensitizing effect of thiazolidinediones is related to increased HMW adiponectin levels (9, 10).

Previously, adiponectin receptors AdipoR1 and AdipoR2 — containing 7-transmembrane domains that are structurally and functionally distinct from G protein-coupled receptors — have been described (8). AdipoR1 is more widely expressed and enriched in muscle, while AdipoR2 is abundant in liver. Binding of adiponectin to AdipoR1 and AdipoR2 increases AMPK activation and PPAR α signaling, resulting in suppression of gluconeogenesis, stimulation of fatty acid oxidation, and amelioration of diabetes (8, 11). AdipoR1- and AdipoR2-mediated signal transduction has been implicated in steatosis, inflammation, and oxidative stress, all key abnormalities associated with obesity and the metabolic syndrome (8).

Sharma et al. found that plasma adiponectin concentration was inversely related to urinary albumin excretion in obese African Americans, a group prone to obesity

and chronic kidney disease (7). Conversely, BMI, blood pressure, lipid levels, and plasma levels of IL-6 and plasminogen activator inhibitor-1 (PAI-1) were not associated with albuminuria in this group. To establish a role of adiponectin in the pathogenesis of albuminuria, Sharma et al. compared wild-type mice with adiponectin-deficient mice. Blood pressure, glucose levels, and lipid levels were not affected in *Ad*^{-/-} mice fed a regular rodent diet; however, albuminuria was significantly higher, worsened with age, and was exacerbated by diabetes. Hydrogen peroxide levels increased in the urine of *Ad*^{-/-} mice, consistent with oxidative stress. Electron microscopic examination revealed segmental fusion of the feet processes of podocytes (interdigitated cells that closely invest the glomerular capillary network in the kidney, as shown in Figures 1 and 2, and act in part as a filter for large macromolecules) in *Ad*^{-/-} mice, although the thickness of the glomerular basement membrane and the structures of endothelial and mesangial cells were not altered by adiponectin deficiency. Albumin permeability was increased in a monolayer culture of podocytes from *Ad*^{-/-} mice, consistent with albuminuria in this model.

The authors showed that AdipoR1 was highly expressed in podocytes (7). AMPK phosphorylation was increased in podocytes by adiponectin or the AMPK activator 5-aminoimidazole-4-carboxamide-1- β -D-ribose nucleoside (AICAR; Figure 2A). The distribution of the tight junction protein zonula occludens-1 (ZO-1) was disrupted in podocytes from *Ad*^{-/-} mice and restored by adiponectin or AICAR treatment. Although the involvement of AdipoR1 in

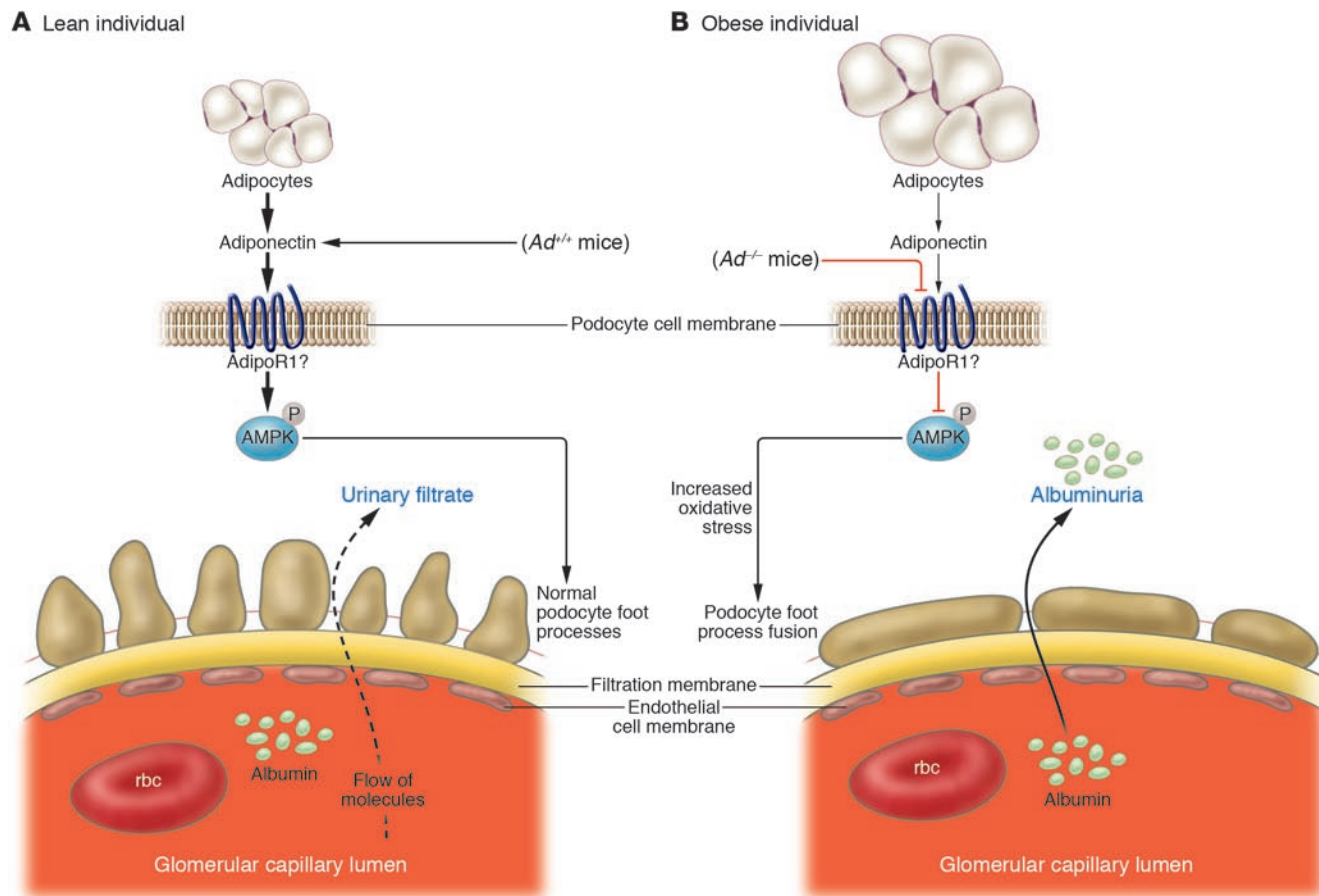
albuminuria and how this is coupled to AMPK signaling requires further study, these results demonstrate major effects of adiponectin on the kidney.

Adiponectin decreases oxidative stress and albuminuria

To determine whether adiponectin is causally related to kidney disease, the authors assessed effects of adiponectin treatment on urine albumin and hydrogen peroxide excretion (7). These parameters were related to podocyte structure, activity of AMPK, and expression of oxidative enzymes. *Ad*^{-/-} mice were treated with the full-length or globular forms of adiponectin. Restoration of plasma adiponectin to levels measured in wild-type mice blunted albuminuria in nondiabetic as well as diabetic *Ad*^{-/-} mice. Remarkably, adiponectin reversed the fusion of podocyte foot processes in *Ad*^{-/-} mice. AMPK activity was attenuated in podocytes and glomeruli, and the expression of the NADPH oxidase Nox4, but not Nox1 or Nox2, was enhanced in the absence of adiponectin in *Ad*^{-/-} mice. As predicted, adiponectin treatment restored AMPK activity and decreased Nox4 expression in parallel with improvements in urinary albuminuria and hydrogen peroxide levels.

Adiponectin as a biomarker of kidney disease: ready for prime time?

These carefully conducted studies demonstrate an important link between adiponectin and albuminuria (Figure 2) (7). The experiments in rodents and cell culture suggest that adiponectin signaling via AMPK regulates oxidative stress, segmental fusion of podocyte foot processes, and albuminuria. The capacity of adiponectin to reverse these abnormalities in *Ad*^{-/-} mice is independent of glucose. These results are in agreement with exacerbation of albuminuria following surgical resection of kidneys in *Ad*^{-/-} mice (12), which resulted in glomerular hypertrophy, podocyte injury, oxidative stress, inflammation, and fibrosis. Adiponectin decreased albuminuria, glomerular hypertrophy, and tubulointerstitial fibrosis, and these changes were associated with restoration of VCAM-1, monocyte chemoattractant protein-1, TNF- α , TGF- β 1, collagen type I/III, and Nox to the levels found in wild-type mice. An antiinflammatory role for adiponectin in the kidney is in agreement with its similar actions in the vasculature, liver, and colon (13–15).

**Figure 2**

Schematic illustration of the effects of adiponectin on podocytes. (A) In lean individuals, high levels of adiponectin phosphorylate and activate AMPK, presumably via AdipoR1, which prevents oxidative stress and the fusion of podocyte foot processes as well as limits albumin excretion. (B) As Sharma et al. demonstrate in this issue of the *JCI* (7), total adiponectin deficiency in *Ad^{-/-}* mice or partial deficiency in obesity diminishes AMPK activity, increases oxidative stress, promotes the fusion of podocyte foot processes, and leads to higher urinary albumin excretion.

Low plasma adiponectin concentration could potentially serve as a biomarker for early detection of kidney disease. However, the inverse relationship between adiponectin and proteinuria among obese African Africans found in the current study (7) has not been consistently observed in others (16, 17). The plasma adiponectin concentration may reflect the extent of kidney damage, metabolic state, and ethnic background of the population under study (7, 15, 16). As mentioned earlier, adiponectin exists as HMW, hexamer, and trimer complexes, yet these forms of the protein were not measured in patients or mice in the current study (7).

The authors' findings in *Ad^{-/-}* mice raise an intriguing possibility that increasing adiponectin levels or stimulating AMPK activity may blunt albuminuria and prevent the progression of kidney disease. However, there are pitfalls in the transla-

tion of these results. Recombinant adiponectin was administered in mice with total adiponectin deficiency. As is often the case in hormone replacement, *Ad^{-/-}* mice are expected to be hyperresponsive to adiponectin treatment. Because there are no patients with total adiponectin deficiency, the question remains as to whether patients with partial adiponectin deficiency will benefit from treatments that increase adiponectin levels. Previous studies in diabetic patients suggest this may be the case: rosiglitazone increased adiponectin levels, enhanced insulin sensitivity, and suppressed albuminuria in patients with type 2 diabetes (18, 19). AMPK activation also has the potential to ameliorate kidney disease, as evidenced by the effects of metformin and AICAR to increase AMPK phosphorylation and inhibit renal hypertrophy in diabetic rats (20).

Conclusions

The article by Sharma et al. (7) advances our understanding of renal pathophysiology. Until the discovery of leptin, the prevailing view of adipose tissue was that of a passive storage site for triglycerides. It is now well established that adipocytes secrete proteins that actively control energy homeostasis, glucose and lipid metabolism, neuroendocrine and cardiovascular function, and various physiological systems. Adiponectin is a logical candidate for exploring the link between adipose tissue and kidney function. The tools for assessing the relationships among adiposity, adiponectin, metabolism, and renal pathophysiology in rodents and humans are readily available, making the clinical translation of key elements of this paper feasible. The demonstration of the validity of adiponectin as a biomarker of albuminuria in humans is a crucial step in



this process and may ultimately facilitate the prevention and treatment of kidney disease.

Address correspondence to: Rexford S. Ahima, University of Pennsylvania School of Medicine, Division of Endocrinology, Diabetes, and Metabolism, 712A Clinical Research Building, 415 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA. Phone: (215) 573-1872; Fax: (215) 573-5809; E-mail: ahima@mail.med.upenn.edu.

1. Ogden, C.L., Yanovski, S.Z., Carroll, M.D., and Flegal, K.M. 2007. The epidemiology of obesity. *Gastroenterology*. **132**:2087–2102.
2. Coresh, J., et al. 2007. Prevalence of chronic kidney disease in the United States. *JAMA*. **298**:2038–2047.
3. Kramer, H., et al. 2005. Obesity and prevalent and incident CKD: the Hypertension Detection and Follow-Up Program. *Am. J. Kidney Dis.* **46**:587–594.
4. Locatelli, F., Pozzoni, P., and Del Vecchio, L. 2006. Renal manifestations in the metabolic syndrome. *J. Am. Soc. Nephrol.* **17**(4 Suppl. 2):S81–S85.
5. de Zeeuw, D., Parving, H.H., and Henning, R.H. 2006. Microalbuminuria as an early marker for cardiovascular disease. *J. Am. Soc. Nephrol.* **17**:2100–2105.
6. Wachtell, K., et al. 2003. Albuminuria and cardiovascular risk in hypertensive patients with left ventricular hypertrophy: the LIFE study. *Ann. Intern. Med.* **139**:901–906.
7. Sharma, K., et al. 2008. Adiponectin regulates albuminuria and podocyte function in mice. *J. Clin. Invest.* **118**:1645–1656.
8. Kadowaki, T. 2006. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* **116**:1784–1792.
9. Pajvani, U.B., et al. 2004. Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J. Biol. Chem.* **279**:12152–12162.
10. Nawrocki, A.R., et al. 2006. Mice lacking adiponectin show decreased hepatic insulin sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor gamma agonists. *J. Biol. Chem.* **281**:2654–2660.
11. Yamauchi, T., et al. 2007. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat. Med.* **13**:332–339.
12. Ohashi, K., et al. 2007. Exacerbation of albuminuria and renal fibrosis in subtotal renal ablation model of adiponectin-knockout mice. *Arterioscler. Thromb. Vasc. Biol.* **27**:1910–1917.
13. Quedraogo, R., et al. 2007. Adiponectin deficiency increases leukocyte-endothelium interactions via upregulation of endothelial cell adhesion molecules in vivo. *J. Clin. Invest.* **117**:1718–1726.
14. Xu, A., et al. 2003. The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver diseases in mice. *J. Clin. Invest.* **112**:91–100.
15. Nishihara, T., et al. 2006. Effect of adiponectin on murine colitis induced by dextran sulfate sodium. *Gastroenterology*. **131**:853–861.
16. Yano, Y., et al. 2007. Differential impacts of adiponectin on low-grade albuminuria between obese and nonobese persons without diabetes. *J. Clin. Hypertens. (Greenwich)* **9**:775–782.
17. Looker, H.C., et al. 2004. Adiponectin concentrations are influenced by renal function and diabetes duration in Pima Indians with type 2 diabetes. *J. Clin. Endocrinol. Metab.* **89**:4010–4017.
18. Pistrosch, F., et al. 2005. Rosiglitazone improves glomerular hyperfiltration, renal endothelial dysfunction, and microalbuminuria of incipient diabetic nephropathy in patients. *Diabetes*. **54**:2206–2211.
19. Miyazaki, Y., Cersosimo, E., Triplitt, C., and DeFronzo, R.A. 2007. Rosiglitazone decreases albuminuria in type 2 diabetic patients. *Kidney Int.* **72**:1367–1373.
20. Lee, M.J., et al. 2007. A role for AMP-activated protein kinase in diabetes-induced renal hypertrophy. *Am. J. Physiol. Renal Physiol.* **292**:F617–F627.

Is Nef the elusive cause of HIV-associated hematopoietic dysfunction?

Frank Kirchhoff¹ and Guido Silvestri²

¹Institute of Virology, University of Ulm, Ulm, Germany.

²Department of Pathology and Laboratory Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, USA.

HIV-associated hematological abnormalities involve all lineages of blood cells, thus implying that the virus impairs the function of early HSCs. However, the underlying mechanisms of this defect are unknown, particularly since HSCs are largely resistant to HIV-1 infection. In this issue of the JCI, Prost and colleagues show that the viral accessory protein Negative factor (Nef) plays a potentially critical role in the pathogenesis of HIV/SIV-associated hematopoietic dysfunction by affecting the clonogenic potential of HSCs (see the related article beginning on page 1765). Soluble Nef induces PPAR γ in uninfected HSCs, thereby suppressing the expression of STAT5A and STAT5B, two factors necessary for proper HSC function. The identification of this novel activity of extracellular Nef defines a new mechanism of HIV/SIV pathogenesis and suggests that approaches aimed at increasing STAT5A and STAT5B expression may be considered in HIV-infected individuals with prominent hematological abnormalities. The results also raise the question of whether dysregulation of hematopoiesis by extracellular Nef plays a role in the development of T cell immunodeficiency and the high levels of chronic immune activation associated with AIDS.

the indirect effects of chronic, generalized immune activation (1). Importantly, HIV infection is also associated with hematopoietic abnormalities characterized by impaired generation and function of cells of all blood lineages (i.e., red blood cells, platelets, and white blood cells). These hematopoietic dysfunctions may cause various levels of cytopenias and also contribute to the development of T cell immunodeficiency (Figure 1) (2). Since all blood cell lineages may be affected, HIV-1 infection apparently deregulates the function of early hematopoietic progenitor cells. This assumption was confirmed by studies showing that CD34⁺ progenitor cells from HIV-infected patients show reduced growth and differentiation in vitro (3, 4).

HIV-associated hematological abnormalities seem to be dependent on the level of virus replication, as these abnormalities are severe in late-stage AIDS patients with high viremia but can be corrected by highly active antiretroviral therapy (HAART) (5, 6). The mechanisms underlying these hematological abnormalities are still obscure. While HSCs express low levels of CD4 and CCR5,

HIV-1 infection causes immunodeficiency by inducing a progressive decline in number of CD4⁺ helper T cells. However, the mechanisms of HIV/AIDS pathogenesis are complex, as it is still unclear to what extent this progressive CD4⁺ T cell depletion is caused by direct killing of infected cells or rather by

Nonstandard abbreviations used: Nef, Negative factor.

Conflict of interest: The authors have declared that no conflict of interest exists.

Citation for this article: *J. Clin. Invest.* **118**:1622–1625 (2008). doi:10.1172/JCI35487.