circulating RAGE ligands is necessary to establish a reliable tool for the assessment of the activity of the RAGE system in human subjects. The study may also be relevant to the role of RAGE and its circulating ligands in other diseases with an activated AGE–RAGE axis, such as carcinomas, Alzheimer's disease, and atherosclerosis. These aggregates may be general phenomena, and the investigation of plasma from patients suffering from these diseases may help to clarify the role of the endogenous ligands.

**DISCLOSURE**

The author declared no competing interests.

**REFERENCES**

1. Selvin E, Steffes MW, Zhu H *et al.* Glycated hemoglobin, diabetes, and cardiovascular risk in nondiabetic adults. *N Engl J Med* 2010; **362**: 800–811.
2. Vlassara H, Brownlee M, Cerami A. High-affinity-receptor-mediated uptake and degradation of glucose-modified proteins: a potential mechanism for the removal of senescent macromolecules. *Proc Natl Acad Sci USA* 1985; **82**: 5588–5592.
3. Schleicher ED, Wagner E, Nerlich AG. Increased accumulation of the glycoxidation product N(epsilon)-(carboxymethyl)lysine in human tissues in diabetes and aging. *J Clin Invest* 1997; **99**: 457–468.
4. Neeper M, Schmidt AM, Brett J *et al.* Cloning and expression of a cell surface receptor for advanced glycosylation end products of proteins. *J Biol Chem* 1992; **267**: 14998–15004.
5. Bierhaus A, Nawroth PP. Multiple levels of regulation determine the role of the receptor for AGE (RAGE) as common soil in inflammation, immune responses and diabetes mellitus and its complications. *Diabetologia* 2009; **52**: 2251–2263.
6. Hofmann MA, Drury S, Fu C *et al.* RAGE mediates a novel proinflammatory axis: a central cell surface receptor for S100/calgranulin polypeptides. *Cell* 1999; **97**: 889–901.
7. Soro-Paavonen A, Watson AM, Li J *et al.* Receptor for advanced glycation end products (RAGE) deficiency attenuates the development of atherosclerosis in diabetes. *Diabetes* 2008; **57**: 2461–2469.
8. Wendt TM, Tanji N, Guo J *et al.* RAGE drives the development of glomerulosclerosis and implicates podocyte activation in the pathogenesis of diabetic nephropathy. *Am J Pathol* 2003; **162**: 1123–1137.
9. D'Agati V, Yan SF, Ramasamy R, Schmidt AM. RAGE, glomerulosclerosis and proteinuria: roles in podocytes and endothelial cells. *Trends Endocrinol Metab* 2010; **21**: 50–56.
10. Penfold SA, Coughlan MT, Patel SK *et al.* Circulating high-molecular-weight RAGE ligands activate pathways implicated in the development of diabetic nephropathy. *Kidney Int* 2010; **78**: 287–295.
11. Andrassy M, Igwe J, Autschbach F *et al.* Posttranslationally modified proteins as mediators of sustained intestinal inflammation. *Am J Pathol* 2006; **169**: 1223–1237.

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# Maximal 'CD80-uria' with minimal change

Andreas D. Kistler[1] and Jochen Reiser[1]

**Differentiation between minimal-change disease and focal segmental glomerulosclerosis remains challenging, particularly at early stages in children and adults. Garin *et al.* identify urinary CD80 excretion levels as a potential marker to differentiate the former entity from the latter. Thus, CD80 expression on podocytes, which was initially described in mouse models of foot process effacement and human lupus nephritis, is now brought toward clinical application for use as a diagnostic biomarker.**

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Minimal-change disease (MCD) is the leading cause of childhood nephrotic syndrome. The disease is ultrastructurally characterized by podocyte foot process effacement without light-microscopic signs of glomerulosclerosis and interstitial fibrosis. Some patients who on initial evaluation are diagnosed as having MCD later develop focal segmental glomerulosclerosis (FSGS), a process of progressive glomerular scarring. It is an ongoing debate whether MCD and FSGS represent different stages of one common pathophysiological process or whether they are different disease entities, with some FSGS patients initially misdiagnosed as having MCD because of biopsy sampling error.

Garin *et al.*[1] (this issue) now demonstrate that urine levels of CD80 may be used to distinguish MCD from FSGS. They have previously shown significantly elevated urinary CD80 levels in pediatric patients with MCD during relapse as compared with MCD in remission, healthy controls, and a small number of patients with other glomerular diseases.[2] They now extend their previous studies by comparing MCD patients with a group of 22 FSGS patients. They were able to show that the elevated urinary CD80 levels are specific to MCD as compared with FSGS patients with similar degrees of proteinuria. Urinary CD80 levels had a high discriminatory value for the two entities, with an area under the receiver operating characteristic curve of 0.99. These findings were corroborated with immunofluorescent stainings of biopsy samples showing high expression of CD80 in glomeruli of MCD patients in relapse but not of MCD patients in remission or of FSGS patients.

CD80—also termed B7-1—is a transmembrane protein expressed on the surface of B cells and other antigen-presenting cells, where it serves as a costimulatory signal for T cells through binding to its receptors CD28 and CTLA-4. Beyond its function as a costimulatory molecule, a novel role for CD80 expressed on podocytes was recently found.[3] CD80 is upregulated in at least two genetic mouse models of glomerular disease, the integrin $\alpha 3^{-/-}$ and the nephrin$^{-/-}$ mouse. In wild-type mice, podocyte expression of CD80 can be induced by the endotoxin lipopolysaccharide (LPS) through toll-like receptor 4 (TLR-4) activation. LPS injection leads to transient podocyte foot process effacement and proteinuria in mice, an effect that is independent of lymphocytes, as it also occurs in SCID mice that are devoid of lymphocytes. CD80-deficient mice are protected from LPS-mediated podocyte injury. Another way to induce CD80 expression in podocytes is treatment with the antibiotic puromycin aminonucleoside. Thus, induction of CD80 is an event common

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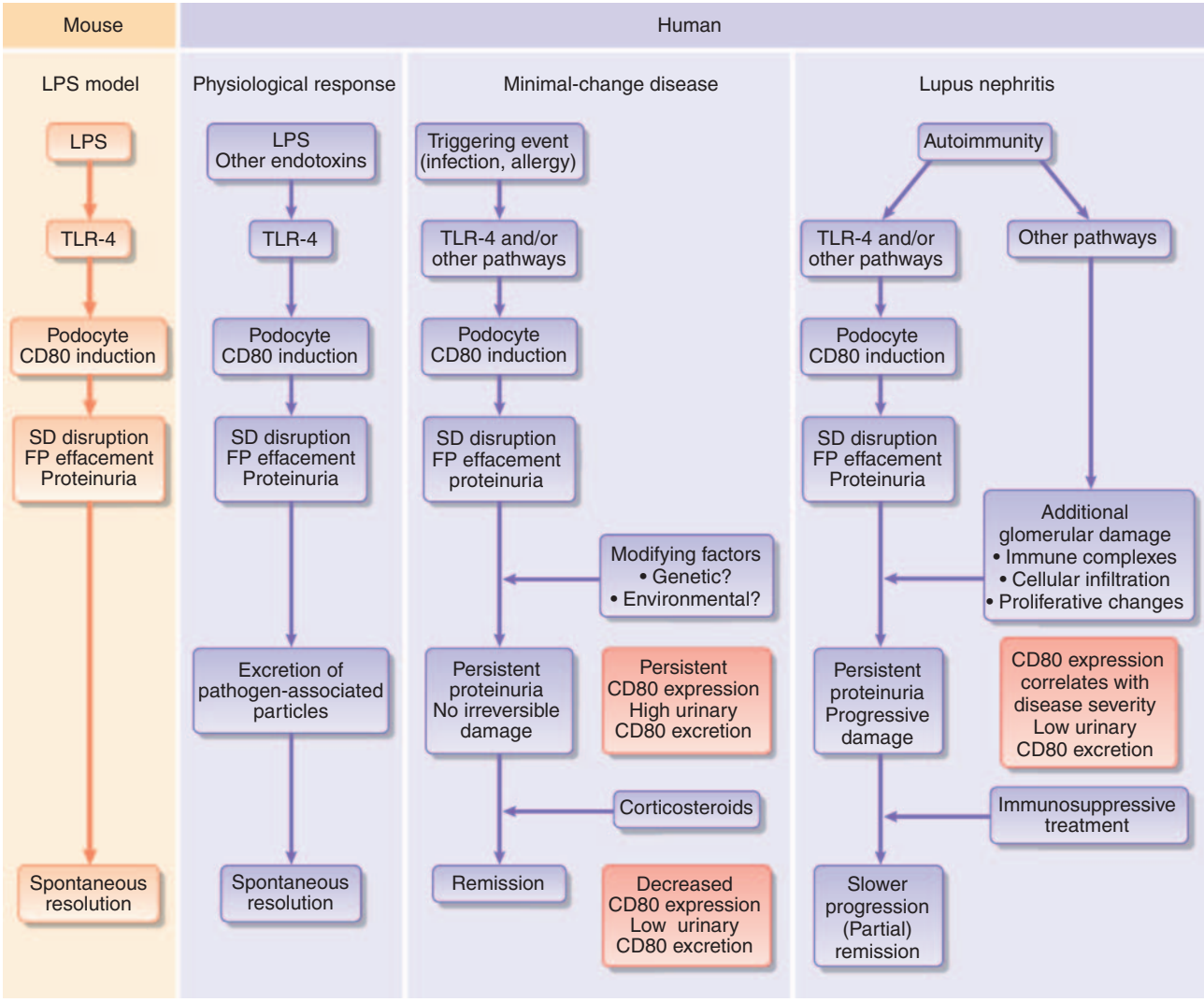


**Figure 1 | The CD80 pathway in the lipopolysaccharide mouse model of proteinuria and its putative physiological and pathophysiological roles in humans.** In mice, lipopolysaccharide injection leads to toll-like receptor 4 activation, CD80 induction, and consecutive slit diaphragm disruption with proteinuria. Proteinuria spontaneously resolves within 72 h of a single lipopolysaccharide injection. In humans, CD80 induction in podocytes may be a physiological response to infections and facilitate the excretion of pathogens by transiently increasing the glomerular permeability to macromolecules. Modifying genetic or environmental factors may lead to persistence of CD80 induction after a triggering event in minimal-change disease (MCD). In lupus nephritis, CD80 induction represents one of several pathophysiological pathways. Podocyte CD80 expression is detectable in both MCD and lupus nephritis and correlates with disease severity in the latter. In contrast, urinary excretion of CD80 is found only in MCD, not in lupus nephritis. FP, foot process; LPS, lipopolysaccharide; SD, slit diaphragm; TLR-4, toll-like receptor 4.

to genetic, immune-mediated, and toxic forms of podocyte injury and seems to be downstream of various signaling pathways. Podocyte expression of CD80 leads to sequestration of the essential podocyte proteins nephrin, CD2AP, and ZO-1, causing disruption of the slit diaphragm complex *in vitro*.[3] The identification of upregulated CD80 in integrin $\alpha 3^{-/-}$ mice further suggests a possible link to integrin signaling.

Structurally, the LPS mouse model of podocyte injury shares key features with MCD in that it exhibits isolated podocyte foot process effacement that is fully reversible. It has therefore been used as an animal model for MCD. It is noteworthy that this structural similarity is now corroborated on the molecular level by the study of Garin *et al.*,[1] justifying the use of the LPS model to identify mechanisms of podocyte injury and potential treatment targets for MCD. One obvious difference, however, is the self-limiting nature of the LPS model in contrast to the prolonged course of MCD. It can be speculated that a physiological role of podocyte CD80 induction is to allow excretion of pathogens during infections.[4] In MCD, the onset of nephrotic syndrome is often preceded by an infection or allergic reaction. This raises the intriguing possibility that MCD results from the prolonged activation of a normally beneficial response (Figure 1). The mechanisms that promote persistence of CD80 expression in MCD, however, remain elusive.

How does CD80 enter the urine? And does urinary excretion of CD80 have a pathophysiological function? In addition to its transmembrane form, CD80 exists as a smaller soluble circulating molecule, which is generated by B cells through

alternative splicing. Thus, Garin *et al.*[1] had to address the question of whether urinary CD80 may arise from filtration of circulating short CD80, as their ELISA-based measurements of urinary CD80 cannot differentiate between soluble and transmembrane CD80. They did so by providing immunoprecipitation–western blot analyses of some urine samples, demonstrating that urine contains full-length CD80. It is therefore unlikely that urinary CD80 arises from glomerular filtration of circulating soluble CD80. By immunostaining of biopsy samples, Garin *et al.*[1] were further able to show CD80 expression in relapsing MCD glomeruli but not in MCD tubules and not in FSGS or MCD in remission. These data provide evidence that urinary CD80 originates from podocytes, although they do not definitely exclude a tubular origin. It is noteworthy that CD80 expression has been shown on tubular epithelial cells of IgA nephropathy patients,[5] raising the possibility that urinary CD80 is derived from tubular epithelium. Assuming that urinary CD80 stems from podocytes, another open question is by which mechanism it ends up in the urine. Urinary CD80 may merely reflect the presence of CD80-positive podocytes lost into the urine,[6] or it may be contained in granular membrane structures that are found in the urine during podocyte injury.[7] Since slit diaphragm proteins have been found to be shed into the urine[8,9] and CD80 binds and sequesters slit diaphragm proteins,[3] CD80 may follow slit diaphragm proteins that are shed into the urine. Alternatively, CD80 may be actively shed from podocytes as a protective mechanism. In this context it is of interest that urinary CD80 levels are not significantly increased in patients with lupus,[2] another disease in which induction of CD80 has been demonstrated in podocytes.[3] One might speculate that active shedding of CD80 protects podocytes from progressive damage in MCD whereas, in severe forms of lupus nephritis, accumulation of CD80 leads to progressive and destructive changes. A more detailed analysis of patient urines, including differential ultracentrifugation to isolate cellular and subcellular urinary contents followed by immunofluorescent and immunoelectron microscopic analysis, would be a first step to clarify the mechanisms of CD80 excretion.

Irrespective of these unanswered pathophysiological questions, urinary CD80 is a promising diagnostic biomarker for MCD. Before getting to clinical use, however, validation in an independent patient cohort is needed, preferably in a multicentric approach. In the study by Garin *et al.* in this issue,[1] the MCD and FSGS patient groups significantly differed in terms of age (mean 6 versus 36 years) and sex (60 versus 18% male); therefore, confirmation in age- and sex-matched patient groups would be desirable. Furthermore, control populations with different kinds of glomerular diseases would be useful to prove the specificity of 'CD80-uria' for MCD. Another important question regarding diagnostic utility is whether patients who are initially classified as having MCD, but later develop FSGS, display low urinary CD80 levels on initial presentation and could thus be identified earlier. Despite these limitations, the study by Garin *et al.*[1] puts us a significant step forward as our quest for glomerular kidney disease markers is ongoing.

## DISCLOSURE

The authors declared no competing interests.

## REFERENCES


1. Garin EH, Mu W, Arthur JM *et al.* Urinary CD80 is elevated in minimal change disease but not in focal segmental glomerulosclerosis. *Kidney Int* 2010; **78**: 296–302.
2. Garin EH, Diaz LN, Mu W *et al.* Urinary CD80 excretion increases in idiopathic minimal-change disease. *J Am Soc Nephrol* 2009; **20**: 260–266.
3. Reiser J, von Gersdorff G, Loos M *et al.* Induction of B7-1 in podocytes is associated with nephrotic syndrome. *J Clin Invest* 2004; **113**: 1390–1397.
4. Reiser J, Mundel P. Danger signaling by glomerular podocytes defines a novel function of inducible B7-1 in the pathogenesis of nephrotic syndrome. *J Am Soc Nephrol* 2004; **15**: 2246–2248.
5. Wu Q, Jinde K, Endoh M *et al.* Clinical significance of costimulatory molecules CD80/CD86 expression in IgA nephropathy. *Kidney Int* 2004; **65**: 888–896.
6. Petermann A, Floege J. Podocyte damage resulting in podocyturia: a potential diagnostic marker to assess glomerular disease activity. *Nephron Clin Pract* 2007; **106**: c61–c66.
7. Hara M, Yanagihara T, Kihara I *et al.* Apical cell membranes are shed into urine from injured podocytes: a novel phenomenon of podocyte injury. *J Am Soc Nephrol* 2005; **16**: 408–416.
8. Gerke P, Sellin L, Kretz O *et al.* NEPH2 is located at the glomerular slit diaphragm, interacts with nephrin and is cleaved from podocytes by metalloproteinases. *J Am Soc Nephrol* 2005; **16**: 1693–1702.
9. Collino F, Bussolati B, Gerbaudo E *et al.* Preeclamptic sera induce nephrin shedding from podocytes through endothelin-1 release by endothelial glomerular cells. *Am J Physiol Renal Physiol* 2008; **294**: F1185–F1194.