

## Recombinant Interleukin-2-Induced Proteinuria: Fact or Artifact?

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Sir,

Induction of proteinuria by recombinant interleukin-2 (rIL-2) is still an open question. Using high-dose rIL-2 in adoptive immunotherapy of metastatic cancers, Rosenberg et al. [1] had suggested that the increase of the vascular permeability was the major adverse effect of this treatment. Thus, rIL-2 could induce proteinuria by its ability to increase vascular permeability. In their attempt to reproduce the vascular leak syndrome (VLS) in mice, Rosenstein et al. [2] demonstrated a strong increase of vascular permeability induced by rIL-2; however, they did not mention excretion of any radiolabelled bovine serum albumin in urine. Similarly, Belldegrün et al. [3] studying specifically the effects of rIL-2 on renal function did not report proteinuria in patients receiving rIL-2 therapy. Recently, Hisanaga et al. [4] reported the onset of nephrotic syndrome (NS) associated with rIL-2 treatment of malignant hemangio-epithelioma. Although these conflicting results may be related to the very different rIL-2 doses used in these studies, the data we reported herein suggest that the commercial source of rIL-2 may be more critical.

In this regard, we have studied the effects of rIL-2 on proteinuria in isolated perfused rat kidney (IPK). Briefly, Wistar rat kidneys, cannulated on renal artery and ureter, were perfused with the Krebs-Henseleit solution (pH 7.4) containing bovine serum albumin (BSA) (2.5 g/l), creatinine (0.03 g/l), and regulated in pressure by gauge manometry. Control or rIL-2 samples were directly injected in the kidney through the renal artery. Urine samples were collected every 5 min. Urinary flow rate, creatinine clearance and proteinuria were measured and the glomerular flow rate (GFR) calculated as the creatinine clearance and the relative proteinuria (RP) as the ratio of proteinuria to the GFR.

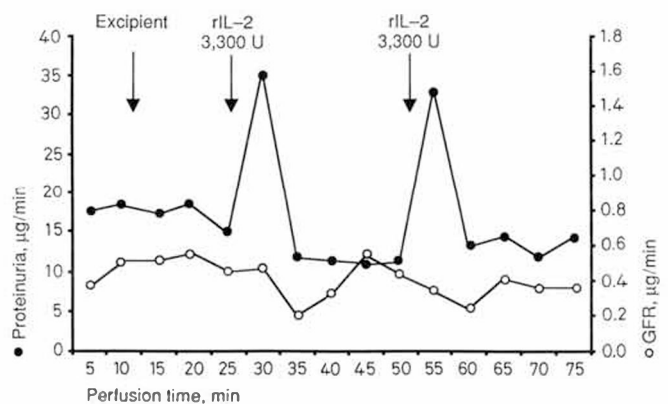


Fig. 1. Time course of proteinuria and GFR into isolated perfused kidney receiving twice 3,300 U of rIL-2 (Janssen).

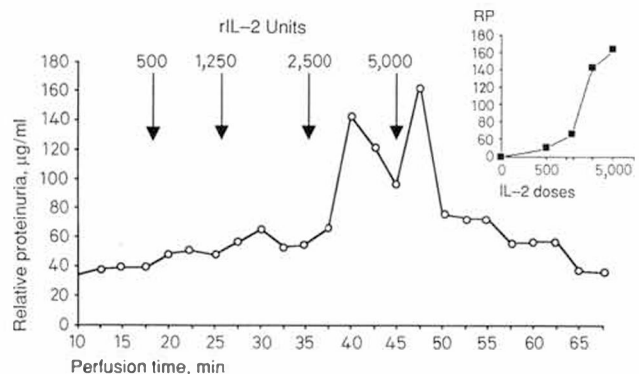


Fig. 2. Time course of relative proteinuria (RP) into isolated perfused kidney receiving increasing doses of rIL-2. Inset: rIL-2 maximal effect on RP.

Injection of 3,300 U human rIL-2 (Janssen, Belgium) increases proteinuria by 3-fold and decreases GFR by 50%. The same rIL-2 injection 30 min later induced quite similar effects (fig. 1). Furthermore, rIL-2 doses ranging from 500 to 5,000 U induced a dose-dependent increase of the RP from 40 to 180 µg/ml (fig. 2). Concomitantly to

the GFR decrease, the perfusion pressure was enhanced, indicating a renal vasoconstriction. All the modifications reversed shortly. A challenge of 9,250 U of rIL-2 for up to 1 h did not alter the renal ultrastructure as assessed by histological studies. Since the rIL-2 Janssen was conditioned in phosphate-buffered saline (pH: 7.4) with BSA (3.6 g/l), the excipient solution consisted of PBS with BSA (Fraction V, Sigma) in the same conditions. Injection of such an excipient as control did not induce any significant modifications. Surprisingly, rIL-2 preparations from other manufacturers (Cetus, Sanofi, Biogen) failed to increase proteinuria even at higher doses.

As indicated by Janssen, rIL-2 contained a low concentration of endotoxins (<0.2 ng/ml). Endotoxins or cytotoxic activity are not possibly responsible for the proteinuric effect since the rIL-2 Janssen is routinely used for the biological IL-2 assay with the CTLL2 cell line. SDS-polyacrylamide gel electrophoresis of the Janssen rIL-2 revealed few high-molecular-weight proteins in addition to the BSA and rIL-2.

Since the rIL-2s from other manufacturers that we have tested failed to induce proteinuria in the isolated perfused rat kidney, we conclude that the proteinuric effect of Janssen rIL-2 is due to contaminants rather than to rIL-2 by itself. Therefore, the mechanism of the VLS, occurring with all the IL-2 preparations as well as with other cytokines, remains unexplained.

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### References

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