Youthful systemic milieu alleviates renal ischemia-reperfusion injury in elderly mice

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The incidence of acute kidney injury (AKI) is high in elderly people, and is difficult to prevent and treat. One of its major causes is renal ischemia-reperfusion injury (IRI). A young systemic environment may prevent the senescence of old organs. However, it is unknown whether a young milieu may reduce renal IRI in the elderly. To examine this question, bilateral renal IRI was induced in old (24 months) mice three weeks after parabiosis model establishment. At 24 hours after IRI, compared to old wild-type mice, the old mice with IRI had significantly damaged renal histology, decreased renal function, increased oxidative stress, inflammation, and apoptosis. However, there was no increase in autophagy. Compared to old mice with IRI, old-old parabiosis mice with IRI did not show differences in renal histological damage, oxidative stress, inflammation, apoptosis, or autophagy, but did exhibit improved renal function. Compared to the old-old parabiosis mice with IRI, the old mice with IRI in the young (12 week)-old parabiosis showed less renal histological injury and better renal function. Renal oxidative stress, inflammation, and apoptosis were significantly decreased, and autophagy was significantly increased. Thus, a youthful systemic milieu may decrease oxidative stress, inflammation, and apoptosis, and increase autophagy in old mice with IRI. These effects ameliorated IRI injuries in old mice. Our study provides new ideas for effectively preventing and treating AKI in the elderly.

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The elderly have a heightened risk of developing acute kidney injury (AKI), and elderly men have a high level of morbidity resulting from the disease.[1–3] A population epidemiology study of AKI in the United States showed that the incidence of dialysis-requiring AKI was 4.5-fold higher in patients older than 65 years of age than in younger patients.[4] Age is considered an independent risk factor for AKI mortality.[5] Compared with younger patients, elderly AKI patients are less likely to recover renal function.[6] Therefore, the prevention and treatment of AKI in the elderly is an important clinical topic.

Parabiosis is an experimental model wherein the muscle and hypoderm of 2 organisms, such as 2 mice, are joined surgically to produce a shared circulatory system. Within this common blood circulation, blood cells and soluble factors can be exchanged continuously. Parabiosis has been used to study the different internal environmental factors that affect organ function and recovery from damage. In recent studies applying the parabiosis model, old mice exposed to a youthful systemic milieu showed rejuvenated progenitor cells, improved cognitive levels, and enhanced remyelination after experimentally induced demyelination.[7–9] Exposure to a young internal environment dramatically reversed age-related cardiac hypertrophy and restored heart function.[10] In addition, old animals exposed to a youthful systemic milieu showed reversal of the aged fracture repair phenotype and a regenerated osteoblastic differentiation capacity.[11] In another study, the muscle mass and ultrastructure of old mice were restored and the muscle regenerative potential was increased after exposure to a young internal environment.[12] In addition, young internal environment exposure has been shown to increase pancreatic beta cell replication,[13] regeneration of aged liver, and renewal of the ovarian follicle reserve.[7,14] To date, no studies have examined the effect of a youthful internal environment on kidney injury in old animals.

Ischemia-reperfusion injury (IRI) is one of the main causes of AKI in the elderly. The mechanism of IRI is related to renal oxidative stress, inflammation, apoptosis, and autophagy.[15,16] Aged kidneys show increased oxidative stress, inflammation, apoptosis, and dysregulation of autophagy.[17] The interaction of these factors is an important mechanism of AKI in older patients.[2] Our previous study showed that after renal IRI, older mice suffered from delayed renal tissue

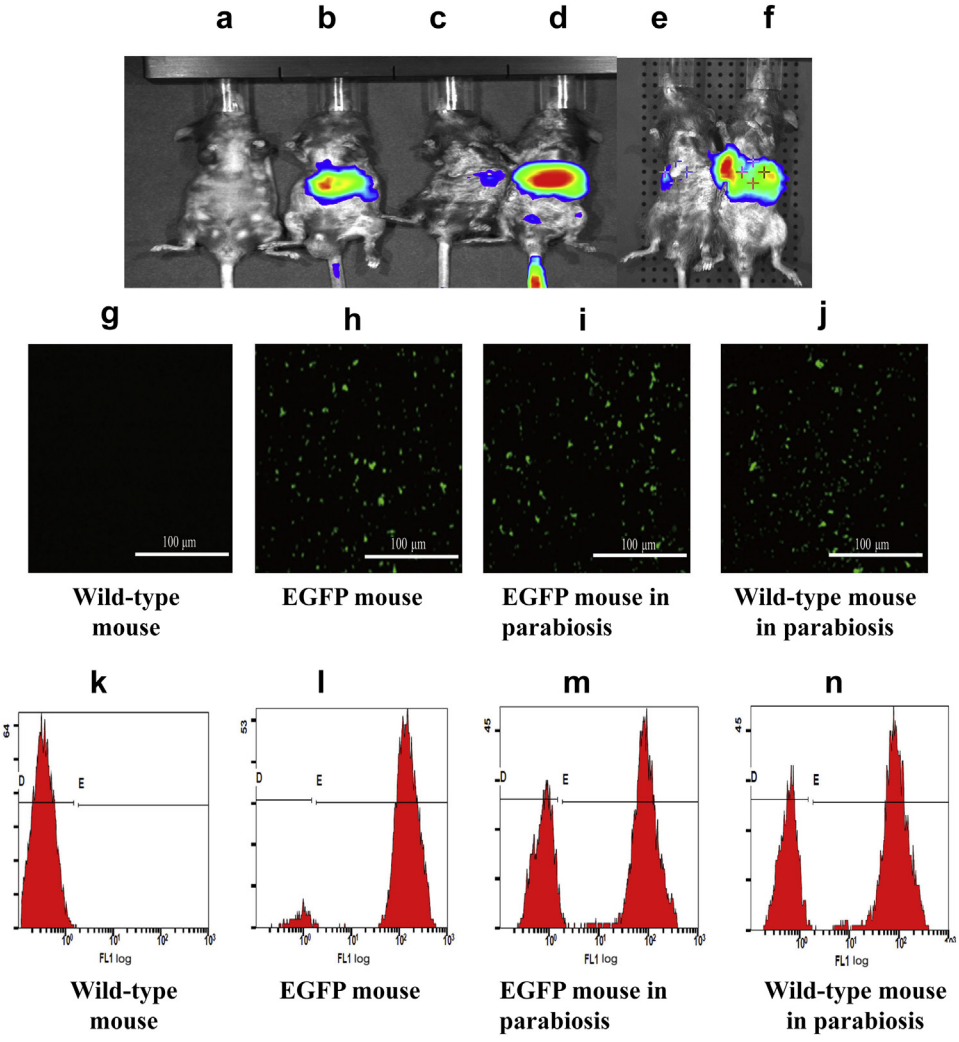

Figure 1 | Verification of the parabiosis mouse model. (**a–f**) Using a small animal *in vivo* imaging system, we observed the body surface positions of fluorescent dyes in mice (n = 3). (**a**) Wild-type mouse injected with phosphate-buffered saline. (**b**) Wild-type mouse injected with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) fluorescent dye. (**c,d**) Parabiosis model. (**c**) After 30 minutes to 2 hours, the distribution of DiR was observed at the spleen's body surface position in the other mouse. (**d**) DiR fluorescent dye was injected into 1 mouse of the parabiosis model. (**e,f**) Parabiosis model. (**e**) After 30 minutes to 2 hours, the distribution of DiR was observed at the liver's body surface position in the other mouse. (**f**) DiR fluorescent dye was injected into 1 mouse of the parabiosis model. (**g–j**) Fluorescence microscopy of peripheral blood smears (n = 3). (**g**) No green fluorescent protein (GFP)–positive blood cells were observed in the peripheral blood smear from the wild-type mouse. (**h**) GFP-positive blood cells in the peripheral blood smear from the enhanced GFP (EGFP) transgenic mouse. (**i–j**) Three weeks after parabiosis was established between the EGFP transgenic mouse and the wild-type mouse, GFP-positive blood cells were observed in the peripheral blood smear of the (**i**) EGFP transgenic mouse and the (**j**) wild-type mouse (original magnification ×200). (**k–n**) Flow cytometry measurements (n = 4). (**k**) The wild-type mice had a negligible amount of GFP-positive cells in the peripheral blood. (**l**) The majority of peripheral blood cells in the EGFP mice were GFP positive. In the parabiosis model, the amounts of GFP-positive cells in the peripheral blood of the (**m**) EGFP transgenic mouse and (**n**) wild-type mouse were similar. To optimize viewing of this image, please see the online version of this article at www.kidney-international.org.

recovery compared with younger mice.[18] In addition, the mortality of the older mice was increased significantly.[18]

Considering these data, we hypothesized that exposing old animals with renal IRI to a youthful systemic milieu would improve their renal IRI. Therefore, we established a parabiosis mouse model with IRI, and observed changes in renal histologic damage, renal function, renal tissue oxidative stress, inflammation, apoptosis, and autophagy versus positive and negative controls. We aimed to explore whether exposure to a young internal environment could improve renal IRI in old mice and to examine the related mechanisms.

RESULTS

Verification of cross-circulation in the parabiosis model with *in vivo* imaging

Two weeks after wild-type parabiosis was established, 1,1′-dioctadecyl-3,3,3′,3′- tetramethylindotricarbocyanine iodide fluorescent dye was injected into 1 mouse in each pair. After 30