DISRUPTED ERYTHROPOIETIN (EPO) METABOLISM IN THE KIDNEYS OF HEREDITARY NEPHROTIC (ICGN) MICE

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Introduction

The ICR-derived hereditary nephrotic mouse (ICGN) was established at the National Institute of infectious Diseases, and is considered to be a good model of nephrotic syndrome [1]. ICGN considered to be a useful model animalin which to examine the pathogenic mechanism of familiar renal diseases and to evaluate the efficacy and/or toxic effects of chemicals. To date, toxicity of environmental pollutants has been assessed using normal healthy animals. However, no information is available concerning the toxicity of environmental pollutants, including environmental endocrine disruptors (Eds), on aged animals or those with hereditary diseases. The ICGN mouse is good model animal in which to evaluate the toxicity of environmental pollutants in patients with renal diseases. Therefore, the present study was performed to investigate the pathogenic mechanism of renal diseases in ICGN mice.

Due to severe renal disorder with unknown etiology, ICGN mice exhibit marked urinary excretion of albumin and other plasma proteins [2]. In the kidneys of ICGN mice, most of the renal tubules are expanded and extracellular matrix (ECM) over-accumulate in the glomeruli and tubulointerstitium [3]. ICGN mice also show irregular cellular kinetics in the kidney [14]. Anemia is a subsequent symptom of chronic renal disease. In recent studies, we have found that ICGN mice develop anemia as the function of the kidney degenerates and we determined the character of anemia in ICGN mice.

Materials and Methods

ICGN mice were prepared at the laboratory of the National Institute of Infectious Diseases, and age- and sex-matched ICR mice were purchased from Clea Japan (Tokyo, Japan). All animals received humane care as outlined in the "Guidelines for the Care and Use of Laboratory Animals "(Kyoto University Animal Care Committee according to NIH No.86-23; revised 1999).

Peripheral blood was collected under ether anesthesia, and then the animals were sacrificed under deep ether anesthesia. The kidney, liver, spleen and testis were rapidly removed and frozen in liquid nitrogen. Hematological and biochemical analyses were performed with a Coulter Counter (K-4500; Sysmex Co., Tokyo, Japan) and an automatic analyzer (Dri-Chem 3500V; Fuji Film Co., Tokyo, Japan), respectively. The concentration of serum erythropoietin (EPO) was determined by amplified EIA technique. EPO mRNA expression in these tissues of ICR and ICGN mice was detected by reverse transcription polymerase chain reaction (RT-PCR). Briefly, total RNA was prepared from liquid nitrogen-frozen tissue samples, using a RNeasy Mini Kit (Qiagen, Chastsworth, CA, USA), and then the total RNA was reverse-transcribed using a Ready-To-Go T-primed First-strand Kit (Amersham Pharmacia Biotech Inc., Piscataway, NJ, USA) to synthesize first strand cDNA, according to the manufacturer's instructions. Then the first strand cDNA prepared from each tissue sample was mixed with the PCR mixture. The following primer pairs specific for partial cDNA sequences of the EPO were used: forward, 5'-TCCTTGCT ACTGATTCCTCTGG-3' and reverse, 5'-AAGTATCCGCTGTGAGTGTTCG-3' to produce a PCR product with the expected size of 152 bp. Mixtures were subjected to PCR on a thermal cycler (GeneAmp PCR Systems 2400; PE Applied Biosystems, Foster City, CA) as follows: PCR conditions for EPO were 40 cycles of 94 °C for 30 min, 57 °C for 30 min, and then 1 cycle of 72 °C for 5 min. PCR products were separated by 2% agarose gel electrophoresis and stained with ethidium bromide solution.

Results and Discussion

With the deterioration of renal function measured by the Creatinine value, ICGN mice developed marked anemia. They showed significantly decreased hematocrit value, hemoglobin concentration and red blood cell count compared to the control ICR mice. Although the mean corpuscular volume (MCV) was decreased slightly (not significantly), the mean corpuscular hemoglobin (MCH) level was not changed, and thus the mean corpuscular hemoglobin concentration (MCHC) was significantly increased. These values indicated that the anemia in ICGN mice is normochromic and normocytic.

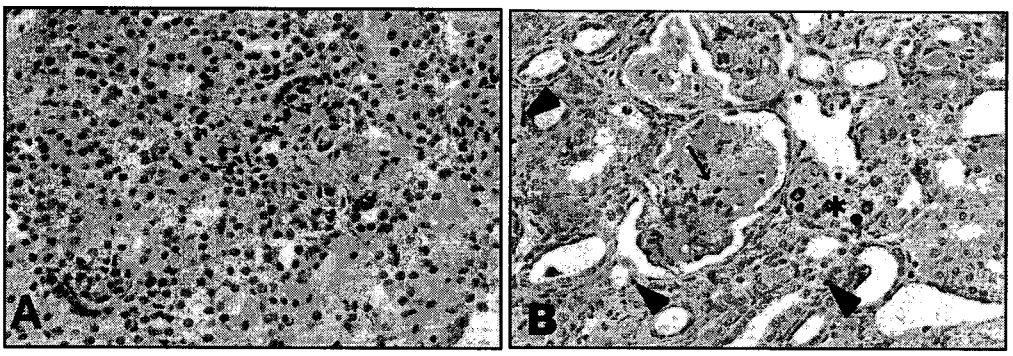

Fig. 1. Kidney sections prepared from 20-week-old ICR (A) and ICGN (B) mice. Proliferating cells were histochemically demonstrated by 5-bromo-2'-deoxyuridine (BrdU) labeling. In ICR mouse sections, no positive reactions for BrdU were demonstrated. In the kidney sections of ICGN mouse, many BrdU-positive cells (proliferating cells) were included in the renal tubular epithelial cells (asterisk), glomerular mesangial cells (arrow) and tublointerstitial fibroblast-like cells (arrowheads). (x 200)

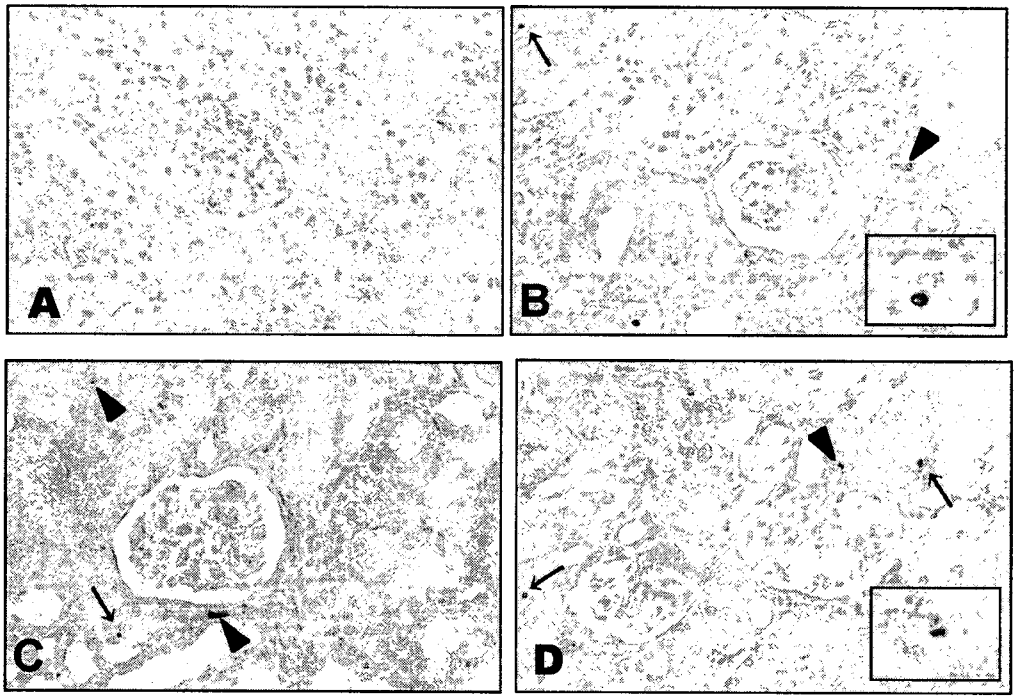

Fig. 2. Kidney sections prepared from 20-week-old ICR (A) and ICGN (B-D) mice. Apoptotic cells were detected by the terminal deoxynucleotidyl transferase-mediated biotinylated deoxyuridine triphosphate nick end-labeling (TUNEL) method. In ICR mouse sections, no positive TUNEL cells were demonstrated. In the kidney sections of ICGN mice, positive TUNEL cells were observed only in the tubulointerstitium cells. TUNEL-positive tubulointerstitium cells were divided into two types; cells with crescent-shaped nuclei

(arrowheads x 200 and thick closed arrow; D, inlet x 800), and those with large round nuclei (arrows x 200 and thick opened arrow; B, inlet x 800).The cells with large round nuclei are considered to be EPO producing cells.

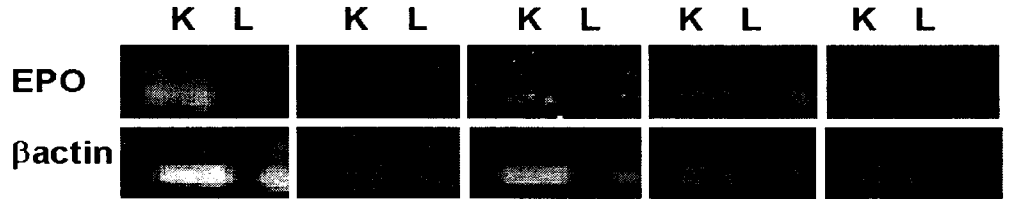

Fig. 3. Representative photographs of RT-PCR products. The higher level of expression of EPO mRNA was detected in liver of ICGN mice.

EPO is a blood-circulating hormone, which controls erythropoiesis. Serum EPO levels in ICGN mice are higher than those in ICR mice, but this level is considered to be insufficient for the degree of anemia. EPO is mainly produced in the kidneys under normal conditions. In ICGN mice, EPO mRNA was expressed not only in the kidney, but also in the liver as determined by RT-PCR. This extrarenal production of EPO suggests that the liver also contributes to the increase of serum EPO level as a substitute production site of EPO.

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