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Original article

IL-33 receptor ST2 deficiency attenuates renal ischaemia–reperfusion injury in euglycaemic, but not streptozotocin-induced hyperglycaemic mice

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ABSTRACT

Aim. – Kidney hypoxia can predispose to the development of acute and chronic renal failure in diabetes. Ischaemia–reperfusion injury (IRI) causes inflammation, and diabetes is known to exacerbate this inflammatory response in the kidney, whereas alarmin IL-33 could act as an innate immune mediator during kidney IRI. Thus, the present study examined the impact of genetic IL-33 receptor ST2 deficiency (ST2−/−) on renal IRI in euglycaemic and hyperglycaemic mice.

Methods. – Hyperglycaemia was induced with streptozotocin (STZ) in adult male C57BL/6JRj wild-type (WT) mice and ST2−/− mice. Unilateral renal IRI was achieved 3 months after STZ treatment by left kidney nephrectomy (non-ischaemic control kidney) and clamping of the right renal artery for 32 min in STZ- and vehicle-treated animals. At 24 h after reperfusion, renal function and injury were determined by levels of plasma creatinine, blood urea nitrogen (BUN) and histological tubule scores. Also, in a complementary pilot clinical study, soluble ST2 concentrations were compared in diabetics and non-diabetics.

Results. – Urinary albumin was significantly increased in STZ-induced hyperglycaemic mice, regardless of genotypic background. At 24 h post-ischaemia, plasma creatinine, BUN and tubular injury were significantly reduced in ST2−/− mice compared with vehicle-treated WT mice, but this protective effect was lost in the STZ-induced hyperglycaemic ST2−/− animals. Plasma concentrations of soluble ST2 were significantly greater in type 2 diabetes patients vs non-diabetics.

Conclusion. – Our data suggest that the IL-33/ST2 pathway exerts differential effects depending on the glucose environment, opening-up new avenues for future research on alarmins and diabetes in ischaemia-related diseases.

Introduction

Diabetes mellitus is a chronic condition with incidences and prevalences that are rapidly increasing globally [1], and chronic renal complications are found in approximately one-third of affected patients [2]. In addition to its impact on long-term complications, diabetes is a risk factor for acute kidney injury (AKI) [3]. Recently, diabetes has also emerged as a risk factor for renal hypoxia, probably linking AKI and diabetes [4]. Renal ischaemia–reperfusion injury (IRI) is an important condition that can act in

Abbreviations: AKI, acute kidney injury; BMI, body mass index; BUN, blood urea nitrogen; GFR, glomerular filtration rate; IRI, ischemia-reperfusion injury; HMBS, HydroxyMethylBilane Synthase; HPRT, hypoxanthine phosphoribosyl-transferase 1; PAS, periodic acid Schiff; RLP5, Ribosomal Protein L5; SD, standard deviation; STZ, streptozotocin; UAE, urinary albumin excretion; VEGF-A, vascular endothelial growth factor A; WT, wild-type.

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conjunction with hyperglycaemia to favour the development of diabetic nephropathy [5]. The link between IRI and diabetes is supported by animal models in which acute transient hyperglycaemia increases renal IRI [6,7]. The relationship between IRI and chronic kidney disease in diabetes is not fully understood, but might involve proinflammatory pathways, as supported by data in obese diabetic rats [8].

The alarmin interleukin (IL)-33, a member of the IL-1 cytokine family, is released by endothelial and epithelial cells during stress and necrosis, and is recognized by the immune system as an endogenous danger signal [8–10]. The recent demonstration that IL-33 contributes to cisplatin-induced AKI [11] supports the hypothesis that IL-33 acts as an innate immune mediator during kidney IRI. Indeed, IL-33 targets the invariant natural killer T (iNKT) cells, NK cells, polynuclear neutrophils and monocytes/macrophages [8,9] involved in the development of kidney IRI [12]. It also binds to both isoforms of the ST2 receptor: the transmembrane isoform (ST2 or ST2L) and the soluble isoform (sST2). In addition, ST2 heterodimerizes with IL-1 receptor accessory protein (IL-1RAcP) and transduces IL-33 signals [9].

The role of the IL-33/ST2 pathway was shown to be important in cisplatin-induced AKI [11], whereas the deleterious impact of diabetes was previously established in renal IRI [6,7]. However, the relationship between alarmins and diabetes is still largely unknown. Thus, the aim of our present study was to evaluate the impact of renal IRI in IL-33 receptor ST2-deficient (ST2−/−) mice in the context of streptozotocin (STZ)-induced diabetes. Plasma concentrations of sST2 in diabetic and control subjects were also determined in a complementary pilot clinical study.

Materials and Methods

Study animals and surgical procedures

Eight- to 12-week-old male wild-type (WT) C57BL/6JRj and mutant (ST2−/−) mice were bred in our animal facility (UFR Medicine, Poitiers) under specific pathogen-free conditions. ST2−/− mice were generated as previously described [13], and backcrossed with mice with the C57BL/6JRj background for 12 generations.

Hyperglycaemia was induced by intraperitoneal injections of either STZ (50 mg/kg/day for 5 consecutive days) or vehicle (sodium citrate buffer), as previously described by the Animal Models of Diabetic Complications (AMDCC) consortium (www.diacomp.org/shared/document.aspx?id=19&docType=Protocol). Animals were screened for blood glucose from samples taken after a 4 h fast from the caudal vein, using a Bayer glucometer, to ensure chronic hyperglycaemia.

IRI experiments were performed in animals at 5 months of age and anaesthetized by isoflurane. Temperature control was set at 37 °C. A flank incision was made and left unilateral nephrectomy was performed, allowing access to renal tissue at baseline (D0). IRI was created in the right kidney by 32 min clamping of the renal pedicle followed by 24 h reperfusion (D1). Blood and renal tissue were then collected. All procedures were performed in accordance with the recommendations of the European Accreditation of Laboratory Animal Care and French institutional ethics committee for experimental animals (CEEA; 122: 2012–06).

Renal function tests

Mice were kept in individual metabolic cages for 24 h urine collection, which was performed 2 days before the surgical procedure. Urinary albumin excretion (UAE) rates were determined in these 24 h urine samples using an immunoturbidimetric method. Urinary and blood glucose levels were determined by a glucose oxidase method. Blood samples were collected from the retro-ocular venous plexus. Creatinine and blood urea nitrogen (BUN) were determined using a modified Jaffe's method and colorimetric method, respectively, with a modular system (Roche Diagnostics Deutschland GmbH, Mannheim, Germany).

Histological preparation

One-half of the kidney was fixed in 4% paraformaldehyde. Paraffin-embedded tissues were stained with periodic acid-Schiff (PAS) and examined under an Olympus BX51 microscope. A renal pathologist, blinded to the experimental conditions, evaluated the presence of acute necrosis in proximal tubular cells according to three criteria-tubular dilatation, cell detachment and loss of brush border, using a semiquantitative scoring system and a numerical scale: 0 = no lesion; 1 = lesions affecting < 25% of kidney sample; 2 = lesions affecting 25–50% of kidney sample; 3 = lesions affecting > 50–75% of kidney sample; and 4 = lesions affecting > 75% of kidney sample [14]. Detection of immune cell infiltrates was carried out on PAS-stained tissue samples.

RNA extraction and real-time quantitative reverse transcription PCR

Total RNA was extracted from mouse kidney tissue using NucleoSpin RNA extraction kits according to the manufacturer's instructions (Macherey-Nagel GmbH & Co. KG, Düren, Germany). RNA (1 mcg) was then retrotranscribed using qScript cDNA SuperMix (Quantabio, Beverly, MA, USA). Quantitative real-time (qRT)-polymerase chain reaction (PCR) was performed with the 2X PerfeCTa SYBR Green SuperMix (Quantabio), using the Rotor-Gene Q cycler (Qiagen, Hilden, Germany). For each primer pair used, PCR efficiency was determined and integrated to calculate the relative mRNA quantity against a standard curve. Six housekeeping genes [β-actin (ACTB), hypoxanthine phosphoribosyltransferase (HPRT) 1, ribosomal protein L5 (RLP5), non-POU domain-containing octamer-binding protein (NONO), hydroxymethylbilane synthase (HMBS) and vascular endothelial growth factor A (VEGFA)] were evaluated to determine the reference gene most suitable for normalizing the Ct value of the IL33 and monocyte chemoattractant protein 1 (MCP1) genes for the same set of kidney samples from euglycaemic and hyperglycaemic WT mice. Results were then normalized against VEGFA as a loading control (identified as the most stable expressed gene according to average expression stability values). Table S1 (see supplementary material associated with this article online) presents the primer sequences and GenBank accession numbers used.

Patients and plasma sST2 quantification

Plasma sST2 concentrations were compared in subjects with and without diabetes. Blood samples were collected at the Poitiers Biological Research Centre (CRB; 0033-00068) in a fasting state and stored at −80 °C until needed. All participants gave their written informed consent, and the Metabolic Diseases Biobank (Biobanque Maladies Métaboliques) project was approved by the local ethics committee (CPP Ouest 3). Type of diabetes was determined by clinical factors (age at diagnosis < 40 years for type 1 [T1D], > 40 years for type 2 [T2D]) and by disease duration before definitive insulin requirement (< 1 ear for T1D, > 2 years for T2D).

Diabetic nephropathy was classified according to urinary albumin concentrations as absent (normoalbuminuria), incipiens (microalbuminuria) and established (proteinuria).

Non-diabetic controls were matched with T2D patients by age (± 5 years) and gender. All had come from either a study of healthy volunteers or the Poitiers Biobank, and were in hospital for non-

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