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Cyclic GMP catabolism up-regulation in MRL/lpr lupus-prone mice is associated with organ remodeling

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ABSTRACT

Production of high titer of antibodies against nuclear components is a hallmark of systemic lupus erythematosus, an autoimmune disease characterized by the progressive chronic inflammation of multiple joints and organs. Organ damage and dysfunction such as renal failure are typical clinical features in lupus. Cell hypermetabolism and hypertrophy can accelerate organ dysfunction. In this study we focus on a specific murine model of lupus, the MRL/lpr strain, and investigated the role of cyclic guanosine monophosphate (cGMP) catabolism in organ remodeling of main target tissues (kidney, spleen and liver) in comparison with age-matched control mice. In MRL/lpr-prone mice, the cGMP-phosphodiesterase (PDE) activities were significantly increased in the kidney (3-fold, $P < 0.001$), spleen (2-fold, $P < 0.001$) and liver (1.6-fold, $P < 0.05$). These raised activity levels were paralleled by both an increased activity of PDE1 in the kidney (associated with nephromegaly) and in the liver, and PDE2 in the spleen of lupus-prone mice. The up-regulation of PDE1 and PDE2 activities were associated with a decrease in intracellular cGMP levels. This underlines an alteration of cGMP-PDE signaling in the kidney, spleen and liver targeting different PDEs according to organs. In good agreement with these findings, a single intravenous administration to MRL/lpr mice of nimodipine (PDE1 inhibitor) but not of EHNA (PDE2 inhibitor) was able to significantly lower peripheral hypercellularity ($P = 0.0401$), a characteristic feature of this strain of lupus-prone mice. Collectively, our findings are important for generating personalized strategies to prevent certain forms of the lupus disease as well as for understanding the role of PDEs and cGMP in the pathophysiology of lupus.

1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by the production of antibodies targeting various components of nuclear compartment of which the nucleosome, spliceosome, and also phospholipids are frequently recognized [1]. Renal failure, thrombosis and neurologic disorders are characteristic clinical features of the illness in its advanced stages. Mesangial cell proliferation appears to play an important role in the pathogenesis of progressive glomerular abnormalities, leading to glomerulosclerosis and kidney dysfunctions [2]. Massive splenomegaly has also been reported in about 10% of the patients [3,4]. Widespread enlargement of the lymph nodes is also common, particularly among children, young adults, and blacks of all ages.

MRL/lpr lupus-prone mice develop clinical symptoms with organomegaly [5]. This mouse strain, which is the most commonly studied mouse model of the disease, bears an autosomal recessive mutation in the gene encoding Fas [6]. The $MRL^{+/+}$ background is responsible for the development of autoimmune kidney disease, and the lymphoproliferation (lpr)/*Fas* mutation converts a mild nephritis into a much severe disease, with a 50% mortality rate at 24 weeks of age [7,8]. Although the molecular defect in this gene is not believed to be a cause for human SLE, this mouse model remains relevant because it recapitulates many biological, immunological and clinical features of the human disease. In particular, some studies indicated that in this strain, the volume of the kidney and spleen increases due to lymphocyte infiltration into inflammatory sites [9,10] as also shown in lupus patients [11,12]. Histology studies of these target organs confirmed the occurrence of both increased numbers of immune cells leading to hyperplasia, and increased size of cells [9,13]. Very little is known, however, concerning cell proliferation and hypertrophy in MRL/lpr mouse organomegaly during

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disease progression. Cell hypermetabolism and hypertrophy may accelerate organ dysfunction [14].

Downstream of cyclic nucleotide synthesis by adenylyl and guanylyl cyclases, cyclic nucleotide phosphodiesterases (PDEs) plays a pivotal role in cyclic adenosine monophosphate (cAMP) and cyclic guanosine monophosphate (cGMP) signaling by regulating protein kinase A (PKA) and protein kinase G (PKG)-dependent phosphorylations, cyclic nucleotide gated (CNG) channels, hyperpolarization-activated cyclic nucleotide-gated (HCN) channels and the exchange protein directly activated by cAMP (EPAC, also known as cAMP-GEF) [15]. This superfamily of enzymes hydrolyzes cAMP and cGMP as a quick feedback mechanism to return to basal levels. In mammals, 11 PDE families are currently known (PDE1–PDE11); most of them consist of several isozymes and numerous PDE isozyme-splice variants. All PDEs share a structural feature of a catalytic core, and they differ in substrate affinity (cAMP and/or cGMP), kinetic properties, specific inhibitor sensitivity and allosteric regulators [15,16]. By regulating cyclic nucleotide levels, PDEs contribute to their compartmentalization and govern many biological processes among them cell adhesion, proliferation and migration. Calcium/calmodulin (Ca^{2+}/CaM) activated-PDE1 has been reported to play an important role in cell proliferation and is implicated in rodent heart hypertrophy [17,18]. Notably, in early cardiac hypertrophy induced by angiotensin II, increases in PDE1 and PDE5 activities associated with increased expressions and in PDE2 activity were reported [18]. In the same way, PDE1 activity and expression increase in cirrhotic rat kidneys after bile duct ligation [19]. Furthermore, we reported earlier in bovine aortic endothelial cells increases of cGMP-hydrolyzing activities related to PDE1, PDE2 and PDE5 when cobblestone (quiescent) phenotype transforms in spindle (angiogenic) phenotype [20], and increases of PDE2 activity and mRNA levels in human vein endothelial cells is stimulated by vascular endothelial growth factor [21]. Chronic renal failure is associated with diminished nitric oxide availably and many of the biological actions of nitric oxide are mediated by cGMP [22].

These observations led us to hypothesize that cGMP-PDEs may play a role in MRL/lpr mouse nephritis and organ remodeling. In this study, we investigated cGMP-PDE mRNA and protein expression activity and location in MRL/lpr mice during disease progression in comparison with age-matched healthy control mice.

2. Materials and methods

2.1. Materials

For PDE activity assessment, cyclic guanosine monophosphate (cGMP), Tris, ethylene glycol tetraacetic acid (EGTA) and erythro-9-(2-hydroxy-3-nonyl) adenine (EHNA) were from Sigma (St. Louis, MO). Nimodipine and 1,3 dimethyl-6-(2-propoxy-5-methane-sulphonylamidophenyl)-pyrazolo[3,4-d] pyrimidin-4-(5H)-one (DMPPO) were generous gifts from Bayer (Berlin, Germany) and Pascal Grondin (Glaxo, Les Ullis, France), respectively. [8-^{3}H] cGMP (30–50 Ci $mmol^{-1}$) was purchased from New England Nuclear (Boston, MA) and purified by thin layer chromatography on silica gel, using isopropanol:NH_4OH:H_2O (70:15:15) as the solvent. Nimodipine, EHNA and DMPPO were dissolved in 100% dimethyl sulfoxide (DMSO). The final concentration of DMSO did not exceed 1% for PDE activity assessment. The rabbit polyclonal antibodies to PDE1A (#PD1A-101AP), PDE1B (#PD1B-201AP), PDE1C (#PD1C-301AP) and PDE2A (#PD2A-112AP) from FabGennix, Frisco, TX, were used for Western immunoblotting and immunohistochemistry analyses. Anti-glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody was from Chemicon (Molsheim, France). Second antibodies used as horseradish peroxidase-conjugates were from Promega (Lyon, France). ECL kit was from GE Healthcare (Uppsala, Sweden). Total RNA was isolated using RNeasy mini Kit (Qiagen, Hidden, Germany) and their expression analyzed by Q-PCR using Biorad kits (iScrpit™ cDNA Synthesis and iQ™ SYBR Green Supermix) in a MyIQ™ thermocycler. Specific primers were obtained from Invitrogen (Table 1).

2.2. Animal model and treatments

Female CBA/J and MRL/lpr mice both sharing the same H-2k MHC haplotype were purchased from Harlan (Gannat, France). Normal mice that display other MHC haplotypes or genetic background were not selected and evaluated in these studies. Mice were maintained in controlled temperature room (25 °C) with a 12 hour-light/dark cycle and were provided with food and water ad libitum. This investigation was carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication no. 85-23, revised 1996). All animal experiments were performed with the approval of the local Institutional Animal Care and Use Committee (CREMEAS).

For measuring in vivo effect of PDE inhibitors on peripheral hypercellularity that develops with time in MRL/lpr mice, 11–13 week-old MRL/lpr mice received either the inhibitors, or a control molecule known to efficiently reduce white blood cell counts, or saline only as non-treated control (single injection; intravenous route; 100 μg molecule/mouse; 8–9 mice per study group). Cell counts were measured five days after injection using a Malassez counting chamber.

2.3. Preparation of kidney, spleen and liver extracts

CBA/J and MRL/lpr mice were sacrificed at 8, 15 and 18 weeks to study PDE activity in relation with lupus progression. At 8 weeks of age, all mice were alive and had no proteinuria (pre-diseased), while at 15 and 18 weeks, 10% and 20% mortality was observed, respectively, and most of mice display significant proteinuria [23]. Kidneys, spleen and liver were isolated, immediately frozen in liquid nitrogen and stored at −80 °C. Frozen tissues were powdered in liquid nitrogen using a mortar/pestle set-up. The resulting tissue powders were homogenized with a glass–glass potter for 3 × 30 s at 4 °C in the following lysis buffer: 20 mM Tris, pH 7.5, 5 mM EGTA, 150 mM NaCl, 20 mM Na-β-glycerophosphate, 1 μM H-89, 10 mM NaF, 1 mM $NaVO_3$, 1% (v/v) Triton X-100, 0.1% (v/v) Tween 20, 166 μM Pefabloc, 133 μM aprotinin, 8.3 μM bestatin, 2.5 μM E64, 3.3 μM leupeptin, and 1.6 μM pepstatin-A. The homogenates were centrifuged at 14,000 *g* for 4 min at 4 °C and the supernatants were stored as aliquots at −80 °C until used. Protein concentration was quantified by the Lowry's method using a compatible detergent assay.

2.4. PDE activity measurements in tissue extracts

PDE activity was determined with a radio-enzymatic assay as described previously [24]. Total cGMP-PDE activity was assessed at 1 μM cGMP and the contributions of PDE isozymes were determined using selective inhibitors: 10 μM nimodipine for PDE1, 20 μM EHNA for PDE2, 10 μM cilostamide for PDE3 and 0.1 μM DMPPO for PDE5. Specific activities were expressed as pmol/min/mg of protein.

Table 1
Primers sequence and amplicon length.

Primers name	Sequence	Amplicon length bp
PDE1A forward	5′ CAC TGG CTC ACT GAA CTG GA 3′	
PDE1A reverse	5′ GAC GTG GTG ATT CTC AAG CA 3′	156
PDE1B forward	5′ TCA AGA ACC TGG ACC TCT GG 3′	158
PDE1B reverse	5′ GGC CTC CAG AAA ACT CAT CA 3′	
PDE1C forward	5′ GGT TCA AGA GCA TCG TCC AT 3′	172
PDE1C reverse	5′ TGA TCT CCA CTG GCC TCA TT 3′	
PDE2A forward	5′ TCC AGA CTG GTG TGT GAG GA 3′	179
PDE2A reverse	5′ CCC AGT AGG TCC ACT TGC AT 3′	