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Autoimmunity to Uroplakin II Causes Cystitis in Mice: A Novel Model of Interstitial Cystitis

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Abstract

Background: The pathophysiology of interstitial cystitis (IC) is unknown. Deficits in urothelial cell layers and autoimmune mechanisms may play a role.
Objective: To examine whether immunization of mice with recombinant mouse uroplakin II (rmUPK2), a bladder-specific protein, would provoke an autoimmune response sufficient to create an IC phenotype.
Design, setting, and participants: RmUPK2 complementary DNA was generated, transferred into a bacterial expression vector, and the generated protein was purified. Eight-week-old SWXJ female mice were immunized with rmUPK2 protein via subcutaneous injection of 200 μg of rmUPK2 protein in 200 μl of an emulsion.
Measurements: Mice were euthanized 5 wk after immunization. Axillary and inguinal lymph node cells were tested for antigen-specific responsiveness and cytokine production, serum isotype antibody titers against rmUPK2 were determined, and gene expression of inflammatory mediators was measured in the bladder and other organs. For functional analysis, mice were placed in urodynamic chambers for 24-h micturition frequency and total voided urine measurements.
Results and limitations: Immunization with rmUPK2 resulted in T-cell infiltration of the bladder urothelium and increased rmUPK2-specific serum antibody responses in the experimental autoimmune cystitis (EAC) mice models compared with controls. The ratio of bladder to body weight was increased in EAC mice. Quantitative reverse transcriptase polymerase chain reaction analysis showed elevated gene expression of tumor necrosis factor α, interferon γ, interleukin (IL)-17A, and IL-1β in bladder urothelium but not in other organs. Evaluation of 24-h micturition habits of EAC mice showed significantly increased urinary frequency ($p < 0.02$) and significantly decreased urine output per void ($p < 0.021$) when compared with control mice.
Conclusions: Our study showed that a bladder-specific autoimmune response sufficient to induce inflammation and EAC occurs in mice following immunization with rmUPK2. EAC mice displayed significant evidence of urinary frequency and decreased urine output per void. Further phenotype characterization of EAC mice should include evidence for pain and/or afferent hypersensitivity, and evidence of urothelial cell layer damage.

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1. Introduction

Interstitial cystitis (IC) is a chronic sterile inflammation of the bladder [1,2], characterized by chronic symptoms of urinary frequency and urgency accompanied by discomfort or pain in the bladder and the lower abdomen, requiring the recent addition of the term *painful bladder syndrome* (PBS) [1,3,4]. IC/PBS primarily affects women, with a female-to-male ratio of 5:1 [1,5]. It is estimated that as many as a million people in the United States are affected by IC [6].

The etiology of IC remains unknown. Potential pathophysiologic causes proposed include inflammatory, neurogenic, autoimmune, vascular, or lymphatic disorders; self-destruction by loss of the glycosaminoglycan layer from superficial cells; and the presence of toxic substances in the urine [7]. IC may have multiple etiologies, all of which result in a similar clinical manifestation. A possible autoimmune etiopathogenesis for IC continues to trigger interest in the scientific community with increasing reports on the association between IC and other autoimmune diseases such as lupus erythematosus, rheumatoid arthritis, ulcerative colitis, and thyroiditis [8,9], and reports of higher incidence of autoantibodies in patients with IC [9]. Whether the chronic inflammation and consequent tissue damage expose bladder tissues to further noxious stimuli and thus eventually lead to an autoimmune response warrants further investigation.

Recently, the use of experimental autoimmunity by the induction of proinflammatory type 1 T-cell responses to targeted self-antigens has contributed to the creation of useful models of autoimmune conditions including autoimmune encephalomyelitis [10], autoimmune myocarditis [11], autoimmune oophoritis [12], and experimental autoimmune cystitis (EAC) that mimic the phenotype of human IC [13]. In our prototypic model we immunized mice with a lyophilized mouse bladder homogenate for induction of bladder autoimmunity. However, this method can induce nonspecific systemic autoimmune complications because the bladder homogenates. In the current study, we aimed to generate an enhanced and more specific EAC model by targeting uroplakin II (UPK2). Uroplakins (UPK 1, 2, and 3) are a family of integral membrane proteins of urothelium [14] highly expressed in bladder tissue. In a transgenic mouse model, UPK2 promoter-driven expression of SV40 induced bladder urothelium cancer specifically [15]. Multiple studies have demonstrated the stability of UPK2 expression in human bladder cancer cell lines [16]; therefore, the durable expression of UPK2 is a good target for the creation of EAC. We postulated that immunization with UPK2 would cause bladder-specific inflammation without any collateral or systemic autoimmune damage. Here we show that immunization of mice with rmUPK2 results in an autoimmune phenotype confined to the bladder that mimics many of the clinical and histopathologic features of human IC.

2. Materials and methods

2.1. Generation and purification of recombinant mouse uroplakin 2

Total RNA was extracted from mouse bladder with TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The complementary DNA (cDNA) was synthesized with random hexamers (Applied Biosystems, Foster City, CA, USA) using M-MLV reverse transcriptase (Promega, Madison, WI, USA). Mouse UPK2 was subcloned into the pET 30b vector (EMD Chemicals, Gibbstown, NJ, USA) using NcoI and HindIII (NEB, Ipswich, MA, USA) restriction enzymes. The cloned vector was transformed into BL21 (DE3) and expressed at 20 °C overnight (approximately 22 h) after the addition of 1 mM isopropyl β-D-1-thiogalactopyranoside. The expressed protein was purified using Ni-column affinity purification under denaturing conditions followed by reverse-phase high-performance liquid chromatography (HPLC) purification to remove any residual endotoxin.

2.2. Mice and immunization

SWXJ (H-$2^{q,s}$) female mice (n = 90) were generated by mating SJL/J (H-2^{s}) males with SWR/J (H-2^{q}) females at Jackson Laboratory (Bar Harbor, ME, USA). At 6–8 wk of age, mice were injected subcutaneously in the abdominal flank with 200 μl of an emulsion of equal volumes of water and complete Freund's adjuvant (CFA) with (EAC mice) or without (control mice) 200 μg of recombinant mouse uroplakin 2 (rmUPK2) protein and 400 μg of *Mycobacteria tuberculosis* H37RA (Difco, Detroit, MI, USA) as previously described [12]. This maximum concentration of rmUPK2 (200 μg) was chosen based on previous studies [12,17]. Mice were euthanized by asphyxiation with carbon dioxide (CO_2) followed by cervical dislocation 5 wk after immunization. All protocols were preapproved by the institutional animal care and use committee of Case Western Reserve University in compliance with the Public Health Service policy on humane care and use of laboratory animals.

2.3. Cell culture and proliferation assay

As described previously [11,12,17,18], to determine immunogenicity, inguinal and axillary lymph node cells (LNCs) were removed from 10 mice 10 d after immunization with rmUPK2 and cultured as a single-cell suspension in 96-well flat-bottom microtiter Falcon plates (BD Biosciences, San Jose, CA, USA) at 3×10^5 cells per well in Dulbecco modified Eagle medium (DMEM) (Mediatech CellGro, Herndon, VA, USA) with 10% fetal bovine serum (HyClone), 5% HEPES buffer, 2% L-glutamine, and 1% penicillin/streptomycin (Invitrogen Life Technologies) added. Then rmUPK2 and recombinant mouse anti-Müllerian hormone (AMH) (as a control) were added in serial 10-fold dilutions to triplicate wells with positive control wells containing 2 μg/ml antimouse CD3 (BD Biosciences). In some experiments, $CD4^+$ and $CD8^+$ T cells were purified from 10-d primed LNC by positive selection using anti-CD4- and anti-CD8-coated magnetic beads and double passage through a MACS LS column using a MidiMACS cell separator (Miltenyi, Auburn, CA, USA). The purified cells were cultured with 5×10^5 gamma-irradiated (2000 rads) syngeneic splenocyte feeders. All cell cultures were incubated at 37 °C in humidified air containing 5% CO_2. After 96 h, wells were pulsed with [methyl-^{3}H] thymidine (1 μCi per well; specific activity 6.7 Ci/mmol; New England Nuclear, Boston, MA, USA) and harvested 16 h later by aspiration onto glass fiber filters.

2.4. Histologic analysis

Bladders were removed and fixed in 10% neutral formalin overnight. Paraffin-embedded tissue was cut into sections 5 mm thick and then stained with hematoxylin and eosin. Gross histologic observations were performed using light microscopy.

2.5. Cytokine enzyme-linked immunosorbent assays

Cytokine concentrations were determined by enzyme-linked immunosorbent assay (ELISA) measurement as described previously [12]. Briefly, 48-h supernatants of 10-d primed LNC were cultured in supplemented