

## INVESTIGATION OF AN ACUTE ROLE FOR NON-NEURONAL CELLS IN PELVIC PAIN AND BLADDER DYSFUNCTION IN A NOVEL MOUSE MODEL OF EXPERIMENTAL AUTOIMMUNE CYSTITIS (EAC)

### Hypothesis / aims of study

Painful Bladder Syndrome/Interstitial Cystitis (PBS/IC) is a chronic, debilitating bladder condition characterized by pain with bladder filling, relief with voiding, frequency, urgency, and nocturia (1). A growing body of clinical evidence strongly suggests that autoimmune events within the bladder play an important role in triggering pathophysiological changes in PBS/IC, thought to involve increased permeability of the stratified epithelial lining of the bladder (called the urothelium) and/or neurogenic inflammation with upregulation of sensory signaling from the bladder to the central nervous system. Recent evidence has shown that spinal cord glial cells (astrocytes and microglia) may be involved in both the development and maintenance of central sensitization in various chronic pain conditions. Central release of the chemokine fractalkine (CX3CL1) by sensory afferents within the dorsal horn of the spinal cord results in selective activation of microglia. Microglial activation (thought to be transient) leads to downstream activation of astrocytes and the establishment of chronic pain through the modulation of neuronal activity and synaptic transmission (2).

Using a validated mouse model of experimental autoimmune cystitis (EAC) (3), we have noted evidence of visceral hyperalgesia and bladder dysfunction, consistent symptoms of PBS/IC, at 10 days following induction of EAC (see Methods). In addition, we noted that there was evidence of bladder urothelial disruption, which would allow the passage of urine into the underlying bladder tissue and potentially play an important role in the observed pathophysiology. In this study, we examined for evidence of pelvic hyperalgesia and bladder dysfunction and correlation with changes in urothelial barrier morphology at the earlier time point of 5 days following EAC induction, in order to shed light on the mechanistic pathways associated with the onset of PBS/IC symptoms. In addition, we investigated for microglial presence and activation in the dorsal horn grey matter of the sacral spinal cord, which receives sensory afferent innervation from the bladder.

### Study design, materials and methods

Autoimmune cystitis was induced by selectively targeting integral membrane proteins, called uroplakins (UPK), specific to the bladder urothelium. A 200  $\mu$ l subcutaneous injection containing 200  $\mu$ g of the peptide sequence UPK3A 65-84 was administered to 9-week-old female BALB/c mice (EAC group; n=6). A second group of mice received 200 $\mu$ l of the vehicle solution; an emulsion of equal volumes of water and complete Freund's adjuvant (CFA) containing 400  $\mu$ g of *Mycobacteria tuberculosis* H37RA (CFA, Difco Laboratories, Detroit, MI) (CFA group; n=6). A third group of untreated animals served as naïve controls (Naïve; n=4).

Bladder function was assessed prior to and at 5-days following treatment by measurement of 24-hour voiding frequency and voiding volume; frequency-volume chart (FVC). Referred visceral hyperalgesia was assessed at 5-days post-treatment, via application of von Frey filaments to the suprapubic and hind-paw regions. Bladders and sacral spinal cord segments were dissected from animals following perfusion-fixation with 4% paraformaldehyde, post-fixed and cryoprotected by incubation in a 30% sucrose solution before being snap frozen on dry ice. Tissue was cryosectioned (6 $\mu$ m) and processed for immunofluorescence. Indication of potential breach in urothelial barrier function was explored using antibodies against cytokeratins (CK) specific for the apical layer of urothelial cells (anti-CK20) and lower epithelial strata (anti-CK17). Microglial presence and morphology in the sacral dorsal horn was explored using anti-Iba1, a protein present in the microglial cell membrane. Resting microglia have numerous cellular processes, while activated microglia exhibit a rounded "amoeboid" morphology, devoid of processes. Fluorophore-conjugated secondary antibodies were used to visualize binding. TO-PRO<sup>3</sup> fluorescent stain (1:5000, Molecular Probes) was used to detect cell nuclei.

### Results

Evidence of early onset of significant pelvic hyperalgesia was noted in the EAC group of animals (Fig.1a-b). Activated microglia was noted in the dorsal horn of EAC-group sacral spinal cord tissue (Fig.2a-b) in comparison with control tissue. In contrast to our findings of significant differences in FVC data at 10-days post-induction of EAC in a separate study (unpublished data; not shown here), bladder dysfunction was not noted in the present study using the earlier time point of 5-days post EAC-induction. Voiding frequency and mean voided volume pre-and post-treatment, exhibited no significant difference in the EAC test group (n=6) as well as the CFA (n=6) and Naïve controls (n=4); ( $P>0.05$ ; paired Student's t-test). In addition breach in barrier integrity by loss of surface urothelial cells was not indicated again in contrast with previous findings of urothelial apoptosis at 10-days EAC (data not shown here).

### Interpretation of Results

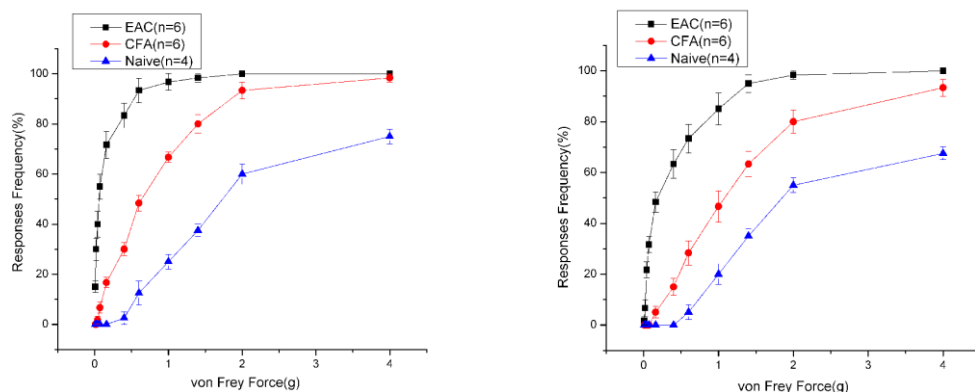
Our data indicate rapid onset of pelvic pain in mice at 5-days post induction of EAC which precedes the onset of bladder dysfunction, seen in turn at a later time point of 10-days post EAC induction. The evidence of microglial activation in the sacral spinal cords of EAC animals indicates the potential for early onset of central sensitisation, laying down the "physiologic" groundwork for the state of chronic pain. Breach in barrier function is indicated to play an important role in onset of bladder dysfunction, seen at 10-days post EAC induction.

### Concluding message

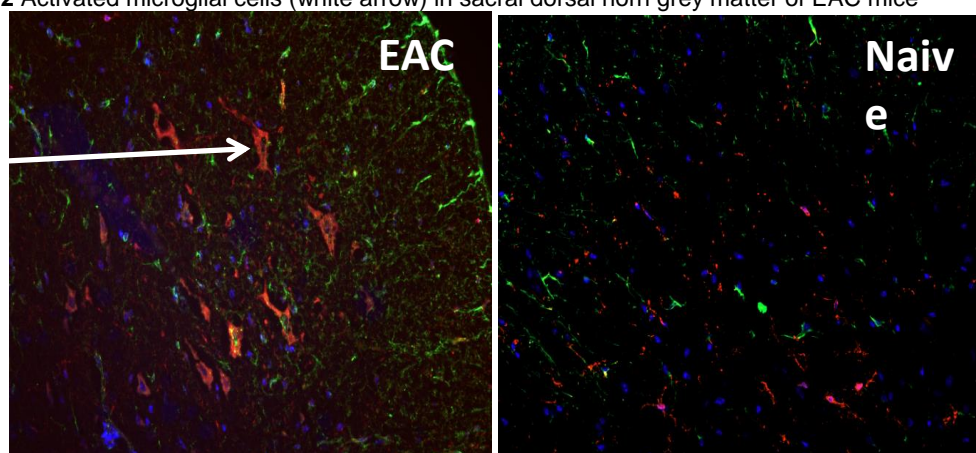
The underlying pathophysiology of PBS/IC is poorly understood and, unfortunately for millions of patients suffering from this debilitating disease, therapeutic strategies for treatment have failed to produce clinically significant results. Characterizing the impact of urothelium-directed autoimmunity on urothelial and spinal cord glial (microglia and astrocytes) physiology will provide

valuable mechanistic information about the underlying pathophysiology of PBS/IC and provide a new prospective on clinical management of this chronic pain syndrome.

**Fig.1 (a):** Suprapubic pain responses at 5 days EAC **(b):** Hind-paw pain responses at 5 days EAC



**Fig. 2** Activated microglial cells (white arrow) in sacral dorsal horn grey matter of EAC mice



#### References

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#### Disclosures

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