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Sex Hormone-Dependent Attenuation of EAE in a Transgenic Mouse with Astrocytic Expression of the RNA Regulator HuR

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Abstract

In experimental autoimmune encephalomyelitis (EAE) and other neurodegenerative diseases, astrocytes play an important role in promoting or attenuating the inflammatory response through induction of different cytokines and growth factors. HuR plays a major role in regulating many of these factors by modulating RNA stability and translational efficiency. Here, we engineered transgenic mice to express HuR in astrocytes using the human glial fibrillary acidic protein promoter and found that female transgenic mice had significantly less clinical disability and histopathological changes in the spinal cord. Ovariectomy prior to EAE induction abrogated the protective effect. Our findings support a role for the astrocyte and posttranscriptional regulation in hormonally-mediated attenuation of EAE.

1. Introduction

HuR, an RNA binding protein, modulates the stability and translational efficiency of many growth factor and cytokine mRNAs by binding to adenine- and uridine-rich elements (ARE) in the 3' untranslated region (UTR) (Barreau et al., 2006; Brennan and Steitz, 2001). This level of gene regulation plays an important role in initiating or terminating inflammatory responses as it can quickly alter expression levels of critical mediators such as TNF- α , COX-2, and interferon- γ (Anderson, 2010). Since the astrocyte expresses a large range of ARE-containing cytokines and chemokine mRNAs that can prolong or attenuate

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inflammation (Dong and Benveniste, 2001; Nair et al., 2008), we sought to determine the impact of HuR transgenic expression on experimental autoimmune encephalitis (EAE). We found significant clinical and histological attenuation of EAE in female and to a lesser extent male HuR transgenic (Tg) mice. Reversal of protection in female mice following ovariectomy indicates the attenuation was hormonally influenced. These findings shed new light on the mechanisms and cell types that contribute to the protective effects of estradiol (E2) or progesterone in EAE.

2. Material and Methods

Mice

The UAB Transgenic Mouse Facility microinjected fertilized eggs of C57BL/6 (B6) mice with a Flag-tagged HuR cDNA (Nabors et al., 2003) construct downstream from the human GFAP promoter (Brenner, 1994). Mice were genotyped using tail clip DNA and the following oligonucleotides: Upstream 5'-TGGACTACAAGGACGACGAT -3', and downstream 5'-CGTCTTTGATCACCTCTGAGC -3'. Mice ages 8–14 weeks of age were used for experiments. All animal studies were performed with approval from the UAB Institutional Animal Care and Use Committee.

Induction of EAE

For active EAE, control and HuR-tg mice were immunized with myelin oligodendrocyte glycoprotein (MOG) peptide_{35–55} as described (Hu et al., 2010). Onset and progression of EAE symptoms were monitored daily (30 days) using a standard clinical scale (Hu et al., 2010). For each mouse, a cumulative disease index (CDI) was calculated from the sum of the daily clinical scores observed between day 7 and day 30. For ovariectomy, mice were sedated using a mixture of ketamine and xylazine. Ovaries were surgically removed and mice were allowed to recover seven days before induction of EAE.

RNA analysis, histology and immunohistochemistry

All mice were sacrificed by CO₂ inhalation. CNS tissues were removed and frozen in OCT (for immunohistochemistry) or liquid N₂ (for RNA analysis). RNA was extracted and reversed transcribed using a reverse transcription kit (Applied Biosystems). PCR was performed with the primers described above. For immunohistochemistry, eight micron transverse sections were cut and briefly fixed with 4% paraformaldehyde (PFA). Sections were blocked, permeabilized and stained with GFAP (DAKO) at 1:1000 and FLAG rabbit polyclonal (Sigma) at 1:5,000 overnight at room temperature. Sections were stained with secondary antibodies, Alexafluor 488 and Alexafluor 594 (Invitrogen) at 1:1000 and DAPI. For EAE histology, mice were sacrificed 30 days post MOG peptide injection. Spinal columns were decalcified and all tissue was paraffin embedded. Five micron sections from the cervical, thoracic and lumbar spinal cord were cut and stained with hematoxylin and eosin or Luxol fast blue and Periodic acid-Schiff. The extent of inflammation, demyelination, and axonal degeneration was scored based on previously published methods (Hu et al., 2010). Briefly, lesions were evaluated on a 0–4 scoring system for inflammation (lymphocyte accumulation and neutrophil infiltration), demyelination, and axonal degeneration without knowledge of the experimental group. Severity scores were calculated as the mean over all segments of the products of the intensity scores multiplied by the extent scores for each lesion characteristic.

3. Results

Generation of the HuR-tg mouse

We used a cDNA containing HuR with an N-terminal Flag epitope (Nabors et al., 2003) and cloned it into a plasmid containing 2.4Kb of the human glial fibrillary acidic protein (GFAP) promoter (Fig. 1A) (Brenner et al., 1994). A positive transgenic line (HuR-tg) was identified by PCR genotyping with a unique primer to the FLAG epitope and a HuR-specific downstream primer. Transgene mRNA expression in spinal cord and brain tissue was confirmed by RT-PCR. Using an anti-FLAG antibody, we detected extensive expression of FLAG-HuR in spinal cord astrocytes (Fig. 1B, upper panel). A predominantly nuclear pattern was observed which is consistent with the cellular distribution of endogenous HuR (Brennan and Steitz, 2001). Colocalization of FLAG and GFAP immunoreactivity was confirmed with confocal imaging (Fig. 1B, lower panel). The transgene was equally distributed throughout the cervical, thoracic and lumbar regions and no gender-specific differences were observed (not shown).

Active EAE is attenuated clinically and histologically in HuR-tg mice

Animals were injected with MOG peptide and then assessed for disease severity using a standard scoring system (Hu et al., 2010). We observed a significant and gender-dependent attenuation of clinical phenotype in HuR-tg mice compared to littermate controls (Fig. 2 and Table 1). In keeping with previously published work, we did not observe a gender effect in wild-type mice (Okuda et al., 2002). Female HuR-tg mice showed significantly delayed disease onset (18.4 vs. 13.4 days for wild-type, $p=0.0035$) and a lower maximum clinical score (2.6 v. 3.8 in wild-type, $p=0.0012$). No clinical signs were detected in 25% of female transgenic mice whereas 100% of littermate controls and HuR-tg males developed disease. HuR-tg males had a significantly lower maximum clinical score ($p=0.01$) and a non-significant trend toward delayed onset and lower cumulative disease index. Ovariectomy of female HuR-Tg mice prior to EAE induction abrogated the protective effect (Fig. 2B). We next evaluated spinal cords of HuR-tg and wild-type mice for histological correlation of this attenuated phenotype (Fig. 2C and D). Spinal cord sections obtained from wild-type mice 30 days after disease induction had significant cellular infiltration in the meninges and white matter with perivascular cuffing and demyelination. For statistical analysis, we scored the degree of inflammation, demyelination, and axonal degeneration in spinal cords of HuR-tg mice and littermate controls using a scale of 0 to 4 based on previously published methods (Hu et al., 2010). For female transgenic mice, there was a significant reduction in the scores of all three histological parameters compared to littermate controls. Male HuR-Tg mice also displayed a significant attenuation in inflammation and axonal degeneration, but not in the degree of demyelination.

4. Discussion

We have described a novel transgenic model whereby expression of the RNA binding protein, HuR, in spinal cord astrocytes produces clinical and histological attenuation of EAE in female, and to a lesser extent, male mice. Reversal of protection after ovariectomy strongly suggests that the effect was modulated by E2, progesterone or both. A possible link between E2 and EAE attenuation was observed in pregnant animals and later confirmed in experiments involving exogenous administration of E2 (Halina and Magdalena, 2006). Progesterone has also been associated with clinical attenuation of EAE (Garay et al., 2007; Yates et al., 2010). Although the protective effect of E2 is dependent on E2 receptor (ER)- α , the identity of effector cell(s) remained unknown until a recent study implicated astrocytes (Halina and Magdalena, 2006; Spence et al., 2011). In that study, investigators observed a loss of protection in EAE when ER- α was conditionally knocked out in astrocytes (Spence

et al., 2011). Astrocytes are immunocompetent cells with pleiotropic effects on CNS inflammation and neuroprotection. Similar to peripheral immune cells, astrocytes can express a broad range of chemokines and cytokines that promote or attenuate inflammation and neuronal injury (Dong and Benveniste, 2001; Nair et al., 2008; Sofroniew and Vinters, 2010). HuR regulates mRNAs of many of these factors by binding AREs in the 3' UTR and uridine-rich elements in the 5' UTR (Abdelmohsen et al., 2008; Barreau et al., 2006; Brennan and Steitz, 2001). HuR modulates two distinct but related levels of posttranscriptional regulation: mRNA turnover and translational efficiency (including internal ribosome entry site-mediated translation) (Abdelmohsen et al., 2008; Durie et al., 2010; Kullmann et al., 2002; Meng et al., 2005; Yeh et al., 2008). Depending on the mRNA target and cellular context, HuR can positively or negatively regulate each level distinctly or in combination. The mRNA target(s) affected by the HuR transgene in our model remains to be characterized. Studies are also ongoing to further characterize the hormonal influence of disease protection (E2 versus progesterone), the role of testosterone, and whether attenuation of male HuR-Tg mice can be enhanced with E2 or progesterone.

In summary, we describe a novel mouse model which links transgenic HuR expression in astrocytes to hormonally dependent attenuation of EAE. This finding provides future direction for understanding the molecular mechanisms and targets for astrocyte-mediated protection in EAE.

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Fig. 1. Generation of the HuR Transgenic mouse

A) HuR cDNA with an N-terminal Flag epitope was cloned into the gfa2 cassette containing ~2.2 kb fragment of the human GFAP promoter (Brenner et al., 1994). Arrows indicate PCR primers that were used for RT-PCR analysis of brain cortex (Ctx) and spinal cord (SC) RNA shown below. RT, reverse transcriptase. B) Upper panel: immunofluorescence of a lumbar spinal cord section from a HuR-tg mouse using anti-Flag antibody (red) and GFAP (green) antibodies with a DAPI counter stain (blue). Lower panels: confocal microscopy with the same antibodies showing colocalization of GFAP and Flag immunoreactivity in a HuR-tg mouse but not a littermate control.

Fig. 2. EAE phenotype is attenuated in HuR-tg mice

A) Clinical assessment of HuR-Tg females (Tg-F), males (Tg-M) and littermate controls (WT) after induction of EAE with MOG peptide. Mice were examined for a total of 30 days. Numbers of mice in each group are shown in parentheses. Results shown are the daily mean clinical score for all groups of mice from three to four independent experiments. See Table 1 for further details. B) Clinical assessment of EAE induction in female HuR-Tg or wild-type mice who received ovariectomy 2 weeks prior to MOG inoculation. C) Representative spinal cord sections from a female HuR-tg and wild-type mouse at 30 days post MOG immunization. Spinal cord sections stained with luxol fast blue and Periodic acid-Schiff (upper panels) show reduced demyelination in HuR-tg female mouse compared to a wild-type (areas of demyelination indicated by arrowheads). H&E staining of the boxed regions (lower panels) demonstrate reduced inflammation in the HuR-tg mouse compared to WT (areas of inflammation indicated by arrows). D) Scoring of spinal cord sections (scale, 0–4; based on 5 μ m thick sections from cervical, thoracic and lumbar regions) for inflammation (InF), axonal degeneration (AxD), demyelination (DeMy). F, female; M, Male. The number of mice examined is shown in parentheses. ** $P < 0.008$, * $P < 0.03$.

Table 1

EAE clinical parameters in wild type, HuR Tg female and HuR Tg male mice.

	CDF^A	Disease Onset^B	Disease Incidence^C	Max Clinical Score^D
WT (n=23)	56.1	13.4d	100	3.8
HuR Tg female (n=12)	30.2 **	18.4d **	75	2.6 **
HuR Tg male (n=13)	47.3	14.1d	100	3.4 *

^A Cumulative Disease Index is the mean of the sum of daily clinical scores observed between days 7 and 30.

^B Disease onset is defined as the first day of two consecutive days with a clinical score of two or more. Onset was significantly delayed in HuR Tg female mice compared to wild type mice.

^C Disease incidence is defined as the percent of mice that displayed any clinical signs of disease.

^D Maximum mean clinical score is the mean of the highest daily clinical score for each mouse. HuR Tg female and male mice had significantly lower scores compared to wild type mice.

**
p < 0.004;

*
p = 0.01