Brain, Behavior, and Immunity 36 (2014) 61–70

Contents lists available at ScienceDirect

Brain, Behavior, and Immunity

journal homepage: www.elsevier.com/locate/ybrbi

Cell-autonomous iodothyronine deiodinase expression mediates seasonal plasticity in immune function

Tyler J. Stevenson [a,*], Kenneth G. Onishi [a], Sean P. Bradley [a,b], Brian J. Prendergast [a,b]

[a] Institute for Mind and Biology, University of Chicago, Chicago, IL 60637, USA
[b] Department of Psychology, University of Chicago, Chicago, IL 60637, USA

ARTICLE INFO

Article history:
Received 6 August 2013
Received in revised form 8 October 2013
Accepted 9 October 2013
Available online 19 October 2013

Keywords:
Thyroid
Photoperiod
Hamster
Cytometry
Rhythm
Adaptive immunity
Imaging flow cytometry

ABSTRACT

Annual rhythms in morbidity and mortality are well-documented, and host defense mechanisms undergo marked seasonal phenotypic change. Siberian hamsters (*Phodopus sungorus*) exhibit striking immunological plasticity following adaptation to short winter day lengths (SD), including increases in blood leukocytes and in the magnitude of T cell-mediated immune responses. Thyroid hormone (TH) signaling is rate-limited by tissue-level expression of iodothyronine deiodinase types II and III (*dio2*, *dio3*), and *dio2*/*dio3* expression in the central nervous system gate TH-dependent transduction of photoperiod information into the neuroendocrine system. THs are also potent immunomodulators, but their role in seasonal immunobiology remains unexamined. Here we report that photoperiod-driven changes in triiodothyronine (T_3) signaling mediate seasonal changes in multiple aspects of immune function. Transfer from long days (LD) to SD inhibited leukocyte *dio3* expression, which increased cellular $T_4 \rightarrow T_3$ catabolism. T_3 was preferentially localized in the lymphocyte cytoplasm, consistent with a non-nuclear role of T_3 in lymphoid cell differentiation and maturation. Exposure to SD upregulated leukocyte DNA methyltransferase expression and markedly increased DNA methylation in the *dio3* proximal promoter region. Lastly, to bypass low endogenous T_3 biosynthesis in LD lymphocytes, LD hamsters were treated with T_3, which enhanced T cell-dependent delayed-type hypersensitivity inflammatory responses and blood leukocyte concentrations in a dose-dependent manner, mimicking effects of SD on these immunophenotypes. T_3 signaling represents a novel mechanism by which environmental day length cues impact the immune system: changes in day length alter lymphoid cell T_3-signaling via epigenetic transcriptional control of *dio3* expression.

1. Introduction

In human and non-human populations, immune function changes markedly over daily and seasonal timescales (Nelson, 2004; Bilbo et al., 2002). Whereas central and peripheral circadian clocks have been identified as key physiological mechanisms responsible for generating diurnal rhythms in immune function (Prendergast et al., 2013a; Logan and Sarkar, 2012; Bechtold et al., 2010), the proximate causes of seasonal cycles in immunity have yet to be identified. Siberian hamsters (*Phodopus sungorus*) are a canonical model for the investigation of seasonal physiological rhythms (Prendergast et al., 2009). Changes in day length (photoperiod) are sufficient to induce widespread alterations in innate and adaptive immunity. Following exposure to short, winter-like photoperiods (SD), hamsters exhibit complete regression of the reproductive system; in parallel, multiple aspects of immune function adopt a winter phenotype: blood leukocyte subpopulations and delayed-type hypersensitivity (DTH) inflammatory responses are enhanced, whereas infection-induced proinflammatory cytokine production, fever, and sickness behaviors are markedly inhibited (Nelson, 2004). Enhancements in blood lymphocyte function and in T cell mediated responses are critical for long-term adaptive immune function during the winter (Nelson, 2004; Walton et al., 2011). Photoperiod-driven changes in nocturnal melatonin secretion are necessary and sufficient for generating seasonal changes in nearly all aspects of immunity studied to date (Yellon et al., 1999; Wen et al., 2007; Freeman et al., 2007); but seasonal changes in gonadal hormone secretion do not fully account for annual cycles in immunity (Prendergast et al., 2005, 2008). The amplitude of seasonal changes in numerous measures of immunity in hamsters encompasses a range that would be clinically diagnostic of an immunocompromised state in humans (Nelson, 2004; Bechtold et al., 2010; Walton et al., 2011), yet hamsters exhibit this reversible seasonal plasticity in the immune system in the absence of comorbid illness.

* Corresponding author. Address: Institute of Biological and Environmental Sciences, University of Aberdeen, Aberdeen AB24 3FX, UK. Tel.: +44 01224 274145.
E-mail address: tyler.stevenson@abdn.ac.uk (T.J. Stevenson).

0889-1591/$ - see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.bbi.2013.10.008

Thyroid hormone signaling plays a central role in photoperiod-driven regulation of reproductive physiology (Yasuo and Yoshimura, 2009). Triiodothyronine (T_3) enhances GnRH signaling to the pituitary via neuro–glial interactions in the median eminence (Yamamura et al., 2004). Systemic levels of the prohormone thyroxine (T_4) do not vary seasonally (O'Jile and Bartness, 1992), but changes in day length markedly alter the expression of iodothyronine deiodinases (DIOs) in the hypothalamus (Yoshimura et al., 2003; Nakao et al., 2008; Barrett et al., 2007; Ono et al., 2008; Prendergast et al., 2013b). Long, summer-like photoperiods (LDs) increase hypothalamic DIO2 expression, which converts T_4 into the receptor-active T_3 and enhances T_3 signaling, whereas SDs increase DIO3 expression, which converts T_4 into the biologically-inactive enantiomer, rT_3, and converts T_3 into T_2, thereby quenching T_3 signaling (Yoshimura et al., 2003; St Germain et al., 2009; Bianco et al., 2002). In sum, photoperiod information is transduced into the reproductive system via DIO-mediated catabolism of T_4, and changes in day length create local thyroid hormone microenvironments in the hypothalamus that effectively gate gonadotropin secretion (Yoshimura et al., 2003).

Thyroid hormones are also potent immunomodulators (Dorshkind and Horseman, 2000), yet a role for T_3 signaling in the genesis of seasonal changes in immunity has not been examined. Thyroid hormone receptors (TRs) are expressed in most lymphoid tissues (Segal and Ingbar, 1982; Foster et al., 1999), and T_3 modulates of lymphoid cell development and immune function (Mascanfroni et al., 2008, 2010). Thymopoesis is markedly reduced in hypothyroid mice (Arpin et al., 2000), as is maturation of B cells (Murphy et al., 1992), both of which are restored by T_4 therapy (Montecino-Rodriguez et al., 1996). Innate immune responses to *Listeria* are suppressed in hypothyroid mice (Foster et al., 2000). In euthyroid mouse and rat models, maturation and activation of antigen-presenting dendritic cells are dependent on non-nuclear (cytosolic) T_3 signaling (Bergh et al., 2005; Mascanfroni et al., 2008, 2010), and supplemental T_4/T_3 treatments enhance T cell-dependent skin immune responses (Chandel and Chatterjee, 1989) and alter mitogen-induced proliferation of blood-, thymus- and spleen-derived leukocytes (Chatterjee and Chandel, 1983).

These experiments examined whether photoperiod-driven changes in thyroid hormone signaling impart seasonal time information into the immune system. Experiments quantified effects of photoperiod on *dio2* and *dio3* mRNA expression in blood leukocytes, and specified a role for epigenetic mechanisms in the regulation of lymphoid cell *dio2*/*dio3* expression by photoperiod. Photoperiod effects on $T_4 \rightarrow T_3$ catabolism and on cellular compartmentalization T_3 were also examined, and *in vivo* experiments tested the hypothesis that T_3 treatment could mimic effects of photoperiod on constitutive and adaptive immunity.

2. Materials and methods

2.1. Subjects

Male and female Siberian hamsters (*P. sungorus*) were selected from a colony maintained at the University of Chicago. Hamsters were housed in polypropylene cages illuminated for 15 h/day (15L:9D). All procedures were approved by the Animal Care and Use Committee at the University of Chicago.

2.2. Study 1: SD induced changes in leukocyte concentrations and mRNA expression

To examine the effects of acute exposure to inhibitory short day lengths (SD; 9 h light/day; lights off at 1700 h CST) male and female hamsters were either housed in the colony LD photoperiod (15L:9D; $n = 13$ M; 11 F) or in SD (9L:15D; $n = 10$ M; 10 F) for 10 weeks. Hamsters were anesthetized with isoflurane gas (4%) and 500 μl whole blood was obtained via the right retroorbital sinus using Natelson tubes coated with sodium heparin. A 25 μl aliquot of blood was mixed with 3% acetic acid at a 1:20 dilution (Unopette, Beckton–Dickinson). Total leukocyte numbers were counted according to methods previously reported (Prendergast et al., 2003). Leukocytes were then isolated from the blood via osmotic lysis followed by DNA/RNA extraction (see below). Gonadal state was assessed as previously described (Prendergast et al., 2013b).

2.2.1. DNA/RNA isolation

Leukocyte DNA and RNA were extracted using QIAGEN AllPrep DNA/RNA mini kit following the manufacturer's instructions. DNA and RNA concentration and quality were determined by spectrophotometer (Nanodrop, Thermo Scientific Wilmington, DE). cDNA was synthesized using Superscript III (Invitrogen, Carlsbad, USA) and genomic DNA and cDNA were stored at −20 °C.

2.2.2. Quantitative PCR (qPCR) for gapdh, dio2, dio3, and dnmts

qPCRs were performed using a BIORAD CFX384 system using the following steps (i) an initial denature at 95 °C for 30 s, then 39 cycles of (ii) 95 °C for 10 s, (iii) annealing dependent on target mRNA (see Table S1) for 30 s and then (iv) an extension at 72 °C for 30 s. The specificity of select samples was established by resolving PCR products in 2.5% agarose gel. A melting curve analysis was added to determine the quality and specificity of each reaction. Quantification of mRNA expression levels was accomplished with iQ Sybr Green Supermix (BIORAD, Hercules, USA). We used PCR Miner (Zhao and Fernald, 2005) to calculate reaction efficiencies (E) and cycle thresholds (CTs). The average E for all reaction was close to 1.0 and samples that were below 0.8 or above 1.2 were excluded from analyses. The expression of each target gene of interest relative to *gapdh* was determined using $2^{-(\text{deltaCT}-\text{deltaCT})}$.

2.2.3. Methylation-sensitive restriction enzyme assay (MSRE)

Leukocyte DNA was subjected to MSRE analyses. The restriction enzymes, *Hpall* and *BstUI* were selected to cleave the *dio3* promoter region at 4 distinct regions within the region amplified during PCR. These enzymes can only cut DNA sequences (CCGG and CGCG, respectively) that are not methylated, and leave methylated DNA intact. Therefore, DNA that is unmethylated will be cut and resulted in lower levels of PCR amplification. 250 ng of genomic DNA was placed into two tubes: an enzyme treated + buffer and a no-enzyme + buffer control. The tubes were then processed using the primers (Table S1) surrounding the targeted CpG sites with the no-enzyme control serving as a reference. 1 μl of each enzyme and 1 μl NEB buffer 1 (New England Biolabs, Ipswich, MA) was added to the tubes, water was added to obtain a final volume of 25 μl. Samples were then incubated at 37 °C for 3 h. Control samples included: no-DNA with enzyme and no-DNA and no-enzyme. *Hpall* and *BstUI* were inactivated by incubation at 65 °C for 20 min. qPCRs were performed using a BIORAD CFX384 system. Samples were run in triplicate. Following and initial denature at 95 °C for 5 min, then 39 cycles of (i) 95 °C for 1 min, (ii) annealing at 61 °C for 1 min and then (iii) an extension at 72 °C for 1 min. A melting curve analysis was added to determine the quality and specificity of each reaction. Quantification of the PCR reaction was accomplished with iQ Sybr Green Supermix (BIORAD, Hercules, USA). Control samples that omitted DNA resulted in no PCR product. We used PCR Miner (Zhao and Fernald, 2005) to calculate reaction E and CTs. The levels of methylation were calculated as a percent methylation where the levels of enzyme treated DNA are expressed as a percent of untreated enzyme DNA using the following equation: (1/(1 + average enzyme treated DNA