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Estrogen Receptor Transrepresses Brain Inflammation

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Estrogen receptors (ERs) have long been implicated in the etiology of multiple sclerosis, but no clear molecular mechanisms have linked ERs to the disease's pathology. Now Saijo et al. (2011) provide evidence that ERβ activates a transrepression pathway that suppresses inflammation and inhibits progression of pathology in a mouse model of multiple sclerosis.

Multiple sclerosis (MS) is a debilitating disease of the central nervous system (CNS). It is characterized by the progressive loss of the myelin sheath that wraps axons and allows efficient communication between neurons. The most prevalent form of the disease is relapsing-remitting MS. Considered an autoimmune disease, relapsing-remitting MS consists of alternative periods of inflammation, in which demyelination occurs, and remission. At the cellular level, relapsing-remitting MS is believed to be driven by three cell types: activated proinflammatory microglia, which are mononuclear phagocytic cells resident in the CNS, and inflammatory T helper 1 (Th1) and Th17 lymphocyte subsets infiltrating the CNS.

The etiological factors contributing to relapsing-remitting MS are not well understood, but they likely involve both genetic and environmental elements. In particular, females suffering from relapsing-remitting MS outnumber males by ~3.21 to 1 in Canada, a ratio that has continued to increase in the past 50 years from ~1.9:1 (Orton et al., 2006). Although the estrogen receptors (ERs) have been implicated in the etiology of MS, no clear molecular mechanisms link them to relapsing-remitting MS. Now, in this issue of *Cell*, Saijo and colleagues may have filled in this gap. They uncover an endogenous mechanism anchored by ERβ, which suppresses neuroinflammation and can inhibit pathologies similar to relapsing-remitting MS in a mouse model of the disease.

Based on the observation that human and mouse microglia express high levels of the ERβ transcript relative to that of ERα, Saijo et al. (2011) first look for synthetic ligands specific for ERβ, which could modulate the inflammatory activity of microglia cells in response to lipopolysaccharide (LPS) in vitro. LPS is an agonist for the toll-like receptor 4 and is often used experimentally to activate the innate immune response in the brain (Figure 1) (Rivest, 2009). The authors find that, from an array of ERβ ligands, only the heterocyclic compounds Indazole-Br and Indazole-Cl consistently inhibit transcription of proinflammatory genes, including inducible nitric oxide synthase *(iNOS)*, interleukin 1β (IL-1β), and IL-23p19. Furthermore, reducing expression of ERβ, but not ERα, completely abrogates Indazole-Cl's inhibition of proinflammatory gene transcription, suggesting that ERβ mediates the repressive effects of the Indazoles.

Saijo et al. next investigate the capacity of Indazole-Cl to inhibit inflammation in the CNS. In mice, experimental autoimmune encephalitis (EAE) recapitulates several pathophysiological hallmarks of relapsing-remitting MS, including progressive paralysis, demyelination, and inflammation sustained by microglia and activated T lymphocytes (e.g., Th17 cells) (Yong and Rivest, 2009). Indeed, Saijo and colleagues find that treating mice with Indazole-Cl is strikingly effective against EAE, both prophylactically and therapeutically. Mice given Indazole-Cl prior to the first signs of paralysis never develop EAE, and the treated mice exhibit reduced infiltration of Th17 cells in the CNS, suggesting that Indazole-Cl successfully impairs the inflammatory response necessary for EAE. Even more remarkable, however, is that treating mice with Indazole-Cl after EAE begins not only inhibits disease progression but also allows for a recovery of the animals' motor functions.

In both the prophylactic and therapeutic experiments, ERβ is absolutely necessary for Indazole-Cl to inhibit EAE; when mice mutant for ERβ (i.e., $ER\beta^{-/-}$ mice) are treated with the drug, they develop EAE similar to nontreated mice. These data strongly suggest that activation of ERβ by Indazole-Cl plays a pivotal role in repressing CNS inflammation.

Next Saijo and colleagues look for endogenous and naturally occurring steroids that bind ERβ and reduce inflammation in the CNS. In particular, the ligand 5-androsten-3β, 17β-diol (ADIOL) can inhibit expression of inflammatory genes by ERβ, and it can be synthesized from its precursor dehydroepiandrosterone (DHEA) within microglia (Jellinck et al., 2007). As reported in previous studies (Du et al., 2001), Saijo et al. (2011) also find that DHEA represses inflammation in EAE mice, although its effects are not as extensive as ADIOL. Furthermore, microglia express relatively high levels of the enzyme 17β-hydroxysteroid dehydrogenase (17β-HSD). 17β-HSD converts DHEA into ADIOL, and inhibiting 17β-HSD renders DHEA ineffective at repressing the transcription of the *IL-6* gene in vitro. Together these data indicate that microglia possess the machinery to produce ADIOL, which can then bind to ERβ in an autocrine/paracrine manner.

Previous studies have described similar anti-inflammatory and/or neuroprotective

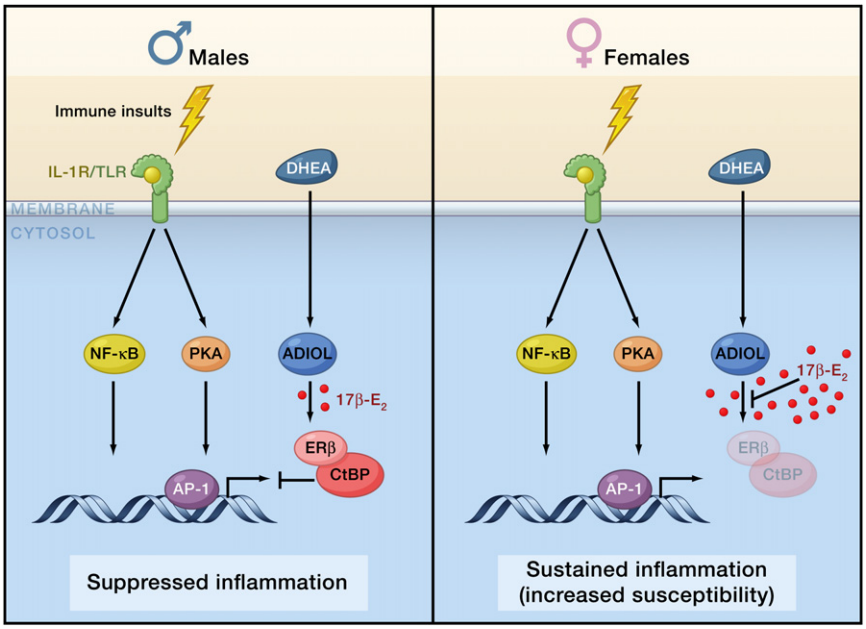

Figure 1. A Possible Model for How Steroid Ligands Influence the Inflammatory Response in Male versus Female Brains

Immune insults trigger proinflammatory signaling pathways in microglial cells through NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) and PKA (protein kinase A), leading to the transcription of innate immune genes. Dehydroepiandrosterone (DHEA), which is present within the cerebral environment, is the precursor of 5-androsten-3β, 17β-diol (ADIOL). ADIOL activates the estrogen receptor beta (ERβ), which then forms a complex with AP-1. The AP1-ERβ complex recruits the corepressor C-terminal binding protein 1 and 2 (CtBP), which shuts off the inflammatory genes. 17β-estradiol (17β-E_2) can antagonize the anti-inflammatory activities of ADIOL, leading to an increase in transcription of proinflammatory genes. If levels of 17β-E_2 are more abundant in female (right) versus male (left) brains, it could shift the equilibrium between these endogenous ligands, resulting in a proinflammatory phenotype. Such a model would help explain why young females are more susceptible than males to multiple sclerosis and possibly other autoimmune diseases.

properties of ER activation and DHEA in EAE (Du et al., 2001; Tiwari-Woodruff and Voskuhl, 2009), but now Saijo and colleagues provide a molecular mechanism for these effects. Specifically, they show that ERβ can act by a "transrepressive mechanism," in which ERβ blocks transcription of proinflammatory genes without binding directly to its target DNA sequence, the estrogen-responsive elements. Instead this repressive mechanism requires AP-1—the transcription factor that contains c-Fos—to bind to promoters of proinflammatory genes (Figure 1). Then the AP-1-ERβ complex recruits the corepressor C-terminal binding protein 1 or 2 (CtBP), which presumably disables the transcriptional machinery (Stossi et al., 2009). Lastly, it is worth noting that this ADIOL-ERβ mechanism promotes only the resolution of inflammation because it interferes with AP-1 only after proinflammatory signals (e.g., LPS, IL-1β) have activated the transcription factor.

The study by Saijo and colleagues raises a number of exciting questions and hypotheses about how ERβ modulates gene transcription in the context of inflammation. Although their data strongly indicate that ADIOL-activated ERβ represses proinflammatory genes by a transrepression mechanism, evidence in the study also suggests that ERβ may directly regulate the initial activation of a subset of proinflammatory genes. For instance, decreasing the expression of ERβ by small inhibitory RNA in human microglia, in itself, diminishes LPS-induced transcription of IL-1β but substantially enhances IL-1β-induced transcription of BAFF (B cell-activating factor) in astrocytes. Moreover, these effects of ERβ occur in the absence of Indazole or ADIOL. Therefore, ERβ appears capable of either augmenting or diminishing the expression of proinflammatory genes in response to danger cues, but how it mediates this differential control is still unknown. Does ERβ directly modulate the chromatin state of promoters or enhancers of immune genes? Or does it indirectly influence basal transcription by interfering with the assembly of corepressors or coactivator complexes. Another intriguing possibility is that ERβ modulates the expression of noncoding RNAs, in particular large intergenic noncoding RNAs, and that these may in return account for the effect of ERβ on the expression of immune genes.

Together the results reported by Saijo and colleagues underline the complexity of the ERβ biology during an inflammatory response and suggest that its effects are context dependent. Thus, the net outcome of ERβ may rest upon an equilibrium that depends on the bioavailability of its endogenous ligands. Indeed, Saijo and colleagues find that the ligand 17β-estradiol (17β-E_2) can antagonize the anti-inflammatory activities of ADIOL, and microglia's innate immune reaction to LPS increases in the presence of 17β-E_2 in vivo (Soucy et al., 2005). Although this makes the net outcome of ERβ activity on gene transcription hard to predict in vivo, it suggests a fascinating mechanism to explain why relapsing-remitting MS affects females more than males. In females, the competition among the steroid ligands for ERβ in an inflammatory context may be shifted to promote inflammation in the CNS (Figure 1). Moreover, anovulants (i.e., birth control medicine) and environmental factors, such as estrogen analogs derived from plants, may also shift the equilibrium of ERβ ligands toward a phenotype that promotes relapsing-remitting MS. These hypotheses clearly warrant further investigations to link mechanistically environmental factors, sex steroids, DHEA metabolism in microglia, neuroinflammation, and chronic diseases.

The study by Saijo and colleagues brings us one step closer to better understanding the critical role that microglia play in sensing the cerebral environment and the resulting consequences of their inflammatory response modulated by nuclear receptors in a gender-specific manner. Compared to the robust anti-inflammatory properties of glucocorticoid receptors, ERβ has not been considered a major modulator of the innate immune

response. This elegant series of experiments by Saijo and colleagues will change this view and hopefully accelerate our understanding of the roles of ERβ in MS and, possibly, in autoimmune diseases more generally, including how gender affects their development.

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Metabolic Homeostasis: HDACs Take Center Stage

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Hormonal regulation of glucose and lipid metabolism is pivotal for metabolic homeostasis and energy balance. Two studies in this issue of *Cell* (Mihaylova et al., 2011 and Wang et al., 2011) introduce a new conserved signaling mechanism controlling catabolic gene expression: class IIa histone deacetylases (HDACs) regulate Foxo activity in *Drosophila* and mice.

The hormones insulin and glucagon are central to regulating glucose homeostasis in vertebrates (Biddinger and Kahn, 2006). During states of fasting, glucagon initiates the production of glucose (i.e., gluconeogenesis) in the liver by increasing the transcription of gluconeogenic enzymes, such as glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxykinase (PEPCK). When nutrients are increased, insulin then attenuates gluconeogenesis in the liver and initiates glucose uptake in peripheral tissues, stabilizing blood glucose concentrations while also promoting anabolic processes and energy storage. When this regulation of glucose homeostasis dysfunctions, metabolic disorders develop, such as type 2 diabetes and metabolic syndrome. Thus, deciphering the details of this signaling network promises to provide new strategies for treating these diseases. Now two studies in this issue of *Cell* take the first step toward bringing this promise to fruition; they uncover a pivotal role for histone deacetylases in glucose homeostasis, suggesting that established HDAC inhibitors may be effective at mitigating diabetes and metabolic syndrome.

In the liver, glucagon stimulates transcription of gluconeogenic genes through a signaling pathway (Figure 1, left) that includes the cAMP-dependent Protein Kinase A (PKA) and the AMP-dependent protein kinase family members AMPK (AMP-activated protein kinase), SIK1 (salt-inducible kinase 1), and SIK2 (Viollet et al., 2009). AMPK and its upstream kinase LKB1 (Liver Kinase 1) inhibit gluconeogenic gene transcription in hepatic cells by suppressing nuclear translocation of the transcriptional coactivator CRTC2 (CREB-regulated transcription coactivator 2)/TORC2. Once PKA blocks AMPK and SIK1/2, CRTC2 associates with the transcription factor CREB to induce the coactivator PGC-1α (peroxisome proliferator-activated receptor gamma coactivator-1 alpha), which in turn associates with the transcription factors Foxo1 (Forkhead box o1) and HNF4α (hepatocyte nuclear factor 4 alpha) to activate gluconeogenic gene expression (Altarejos and Montminy, 2011; Viollet et al., 2009).

Insulin attenuates glucose production by influencing the same pathway (Figure 1, left). Activation of the insulin-responsive Akt kinase results in the inhibition of CRTC2 via SIK1/2, as well as phosphorylation and inactivation of Foxo (Viollet et al., 2009).

In addition to this hormone-mediated control of glucose metabolism, the cell's energy balance also influences glucose production through a family of class III histone deacetylases (HDACs), called Sirtuins, which couple deacetylation with NAD (nicotinamide adenine dinucleotide)