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Pituitary dendritic cells communicate immune pathogenic signals

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ABSTRACT

This study reveals the presence of dendritic cells (DCs) in the pituitary gland, which play a role in communicating immune activation to the hypothalamic pituitary adrenal (HPA) axis. Using enhanced yellow fluorescent protein (eyfp) expression as a reporter for CD11c, a marker of DCs, we demonstrate anatomically the presence of CD11c/eyfp+ cells throughout the pituitary. Flow cytometric analysis shows that the predominant cellular phenotype of pituitary CD11c/eyfp+ cells resembles that of non-lymphoid DCs. *In vivo* and *in vitro* immune challenge with lipopolysaccharide (LPS) stimulates these pituitary CD11c/eyfp+ DCs, but not eyfp^{neg} cells, to increase levels of pro-inflammatory cytokines, IL-6, IL-1β, and TNF-α. *In vivo* analysis of plasma glucocorticoid (GC) and adrenocorticotropic hormone (ACTH) levels at this early phase of the immune response to LPS suggest that pro-inflammatory cytokine production by DCs within the pituitary may activate the release of GCs from the adrenals via ACTH. Pituitary CD11c/eyfp+ cells also express annexin A1 (ANXA1), indicating a role in GC signal attenuation. In summary, our data demonstrate that a resident DC population of the pituitary gland coordinates GC release in the early phase of systemic immune activation, thereby providing an essential immune signaling sentinel for the initial shaping of the systemic immune response to LPS.

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1. Introduction

The pituitary gland is a critical component of neuroendocrine pathways involved in communications between the hypothalamus and the endocrine system and consists of three lobes: anterior, intermediate, and the posterior (Amar and Weiss, 2003). The anterior pituitary is composed of both hormone and non-hormone producing cells, and among the hormone-producing populations are corticotrophs, somatotrophs, gonadotrophs, thyrotrophs, and lactotrophs. Corticotrophs play a pivotal role in maintaining glucose homeostasis, and can be mobilized as part of the body's response to stress and immune challenge (Dallman et al., 1993) by releasing adrenocorticotropic hormone (ACTH). In turn, ACTH stimulates the production of glucocorticoids (GCs) from the adrenal gland (Buckingham, 1970). GCs have been proven to play important roles in honing the immune response, reducing sickness behavior, and preventing septic shock-induced mortality (Kapcala et al., 1995; McEwen et al., 1997).

Among the non-hormone producing cells of the pituitary gland are the folliculo-stellate (FS) cells, which comprise 5–10% of the anterior pituitary and are important for the stabilization of the cellular network (Allaerts and Vankelecom, 2005; Denef, 2008). FS cells, characterized in the rat by S100b expression, are heterogeneous, containing a population of cells that express monocytic markers, such as CD11b and F4/80, as well as MHC-II (Denef, 2008). Functionally they have been shown to perform steady state tasks such as phagocytosis (Allaerts et al., 1991; Denef, 2008; Devnath and Inoue, 2008).

In the immune system, DCs are distinguished by their potent antigen-presenting capacity and their pivotal role in both innate and adaptive immune responses (Steinman, 1991). In the periphery they are found in the circulation with subtypes localized to several immune organs and other tissues. Additionally, data from our laboratory have established the presence of brain DCs in distinct areas of the steady state CNS using the *Itgax* (CD11c) EYFP+ transgenic (Tg) mouse (Bulloch et al., 2008) and further demonstrate their functionality in different models of neuroinflammation and aging (D'Agostino et al., 2012; Felger et al., 2010; Gottfried-Blackmore et al., 2009; Kaunzner et al., 2012). In the current study, using this same transgenic mouse, we identify the presence of CD11c/eyfp cells in the pituitary during steady state and following lipopolysaccharide (LPS) challenge.

It is well established that part of the immune response to LPS is the release of ACTH from corticotrophs in the anterior pituitary, which subsequently stimulates GC secretion from the adrenals

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(Givalois et al., 1994). Hypothalamic regulation of LPS-induced ACTH and subsequent GC release is not required, as cutting the pituitary stalk does not eliminate the LPS-induced activation of pituitary adrenal communication. Furthermore, LPS has been shown to directly stimulate interleukin-6 (IL-6) release from cloned FS cells and potentiate the corticotropin-releasing hormone (CRH)-induced release of ACTH from corticotrophs (Mehet et al., 2012). In addition, LPS is reported to induce hypothalamic pituitary adrenal (HPA) axis activity through an alternative pathway regulated by IL-1, which induces ACTH release in human corticotroph cultures (Haedo et al., 2009). There is evidence that IL-1 acts upstream of IL-6, because inactivating the IL-1 receptor inhibits LPS-induced release of both IL-6 and ACTH (Turnbull, 2003). While cytokines such as IL-1β and TNF-α have been detected in the pituitary, their source remains uncertain (Haedo et al., 2009).

FS cells have been further shown to be involved in the mediation of negative feedback of GCs on ACTH release. Annexin A1 (ANXA1) is a GC-inducible phospholipid binding protein that is expressed by S100b positive FS cells in the anterior pituitary (Traverso et al., 1999). ANXA1 acts in a paracrine manner to inhibit ACTH release and is considered a potential modulator of the anti-inflammatory effects of GCs (Buckingham et al., 2006). Collectively these data suggest that LPS stimulates the production of IL-6 (directly or via IL-1) in the pituitary, which induces ACTH release. In a related loop, GCs also negatively feeds back on ACTH production via ANXA1 in the pituitary.

While there is currently strong evidence for the role of FS cells in neuroendocrine immune interactions, many of the studies that focus on FS cell lines ignore the heterogeneity of FS cells in the pituitary. Several studies have suggested the presence of a dendritic cell (DC)-like subpopulation among FS cells that participates in the interaction between the endocrine and immune systems via signaling pathways and paracrine effects (Allaerts et al., 1996, 1997, 1991). Our studies in the past have highlighted the utility of working with the transgenic mouse line *Itgax* (CD11c) EYFP+ to map the distribution of the DCs in the CNS, as well as characterize their functional phenotype (Bulloch et al., 2008; D'Agostino et al., 2012; Felger et al., 2010; Gottfried-Blackmore et al., 2009; Kaunzner et al., 2012). Using the aforementioned strategies, we herein address the presence and function of a putative DC population within the pituitary and establish its role in neuroendocrine-immune function during a systemic inflammatory immune response. Our findings support the hypothesis that DCs are the primary source of pro-inflammatory cytokines in the pituitary, which in turn mediate the release of ACTH following endotoxin challenge.

2. Materials and methods

2.1. Itgax (CD11c) EYFP+ Tg mice

The Tg mouse line *Itgax* (CD11c) EYFP+ was developed at The Rockefeller University and has been well characterized for its utility in identifying DCs in lymphoid tissues (Lindquist et al., 2004) and in the brain (Bulloch et al., 2008). Female mice were used at 3–4 months of age and were treated in accordance with protocols approved by the Institutional Animal Care and Use Committee at The Rockefeller University.

2.2. Perfusion and tissue collection

Tissue collected for light and fluorescent microscopy was harvested from mice that were deeply anesthetized with sodium pentobarbital (150 mg/kg, intraperitoneally (i.p.), Sigma Aldrich, St. Louis, MO) followed by perfusion with a solution of 4% paraformaldehyde as previously described (Bulloch et al., 2008). Pituitary glands were collected, postfixed, cryoprotected in 30% sucrose solution, and embedded into Optimal cutting temperature (OCT) reagent (Sakura Finetek, Torrance CA). Tissue was cut horizontally on a Leica CM3050S cryostat at 20 μm (Leica Biosystems, Buffalo Grove, IL). Slices were collected on superfrost/plus slides (Thermo Fisher Scientific, Waltham, MA) and stored at −20 °C.

2.3. Fluorescence immunohistochemistry (IHC)

Slide-mounted horizontal sections were processed for fluorescence IHC as previously described (Bulloch et al., 2008). Briefly, horizontal sections were blocked in 0.5% BSA followed by incubation in a primary antiserum overnight at 4 °C. Sections were then incubated in the appropriate secondary antibodies at an experimentally tested concentration for 1 h at room temperature (RT) (see Supplementary Table 1). The secondary antibodies were fluorescence-tagged Alexa-Fluor 647 (Life Technologies, Grand Island, NY) to prevent overlap artifact with the EYFP signal (514 nm). Control sections of pituitary glands were processed without exposure to the primary antibody. For IHC, anti-Annexin-1 (sc-1923 Santa Cruz, Dallas, TX), anti-CD11c (MA1-80129 Thermo Fisher Scientific), and anti-MHC-II (14-5321-32 eBioscience, San Diego, CA) primary antibodies were used (Supplementary Table 1). Fluorescence-labeled sections were imaged with a Nikon Eclipse 90i epifluorescent microscope (Nikon, Melville, NY) or an inverted TCS SP5 laser scanning confocal microscope (Leica Biosystems) at the Rockefeller Bioimaging Resource Center.

2.4. Single-cell pituitary suspension

Mice were rapidly decapitated and pituitaries were extracted under sterile conditions. Due to the small size of the mouse pituitary, it was not technically feasible to harvest tissue from each pituitary lobe. The pituitaries were incubated with collagenase D (Roche, Indianapolis, IN), dispase II (Roche), and DNase (Life Technologies) to yield single-cell suspensions. Cells were then filtered through a 100 μm filter and rinsed in ice cold HBSS.

2.5. Fluorescence-activated cell sorting (FACS) and flow cytometry

A single-cell suspension of pituitary cells was prepared from freshly harvested whole pituitaries from *Itgax* (CD11c) EYFP+ mice, washed with PBS, and stained with DAPI (Sigma Aldrich) to eliminate dead cells. Live eyfp+ or eyfp^{neg} cells were then sorted in a FACS Aria Flow Cytometer (BD Bioscience) at the Rockefeller Flow Cytometry Resource Center. Phenotypic characterization of CD11c/eyfp+ cells was performed on single-cell suspension of pituitary cells, washed and blocked with anti-mouse CD16/32 Fc block (eBioscience), and stained for Molecular Probes Live/Dead Fixable Aqua (Life Technologies). Antibodies were purchased from different sources and used at experimentally determined concentrations as described in Supplementary Table 2. Cells were fixed with the BD Fix/Perm Kit (BD Bioscience). Fixed cells were analyzed on a BD LSR-II flow cytometer (BD Bioscience) with FACS Diva software. Data analysis was conducted using FlowJo software (TreeStar, Ashland, OR). Mean Fluorescent Intensities (MFI) were determined for each antibody and the ratio of MFI to isotype was used to designate cells "+", "low", or "neg".

2.6. In vivo LPS studies

Mice were injected with LPS (5 μg/gbw, i.p.; L3012 Sigma Aldrich) or vehicle (PBS) and sacrificed 1 h post-inoculation (hpi). Pituitaries were removed, dispersed, and sorted as above. Cells were sorted directly into TRIzol reagent (Life Technologies) and

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