General and Comparative Endocrinology 267 (2018) 193–201

Contents lists available at ScienceDirect

General and Comparative Endocrinology

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

Endocrine-immune signaling as a predictor of survival: A prospective study in developing songbird chicks

Emily E. Virgin[a,b,1], Kimberly A. Rosvall[a,b,*]

[a] Department of Biology, Indiana University, Bloomington, IN 47405, USA
[b] Center for the Integrative Study of Animal Behavior (CISAB), Indiana University, Bloomington, IN 47405, USA

ARTICLE INFO

Keywords:
Acute phase response
Corticosterone
Cytokine
Ecoimmunology
Interleukin

ABSTRACT

Immune function varies with an animal's endocrine physiology and energy reserves, as well as its abiotic and biotic environment. This context-dependency is thought to relate to adaptive trade-off resolution that varies from one context to the next; however, it is less clear how state- and environmentally-dependent differences in endocrine-immune signaling relate to survival in natural populations. We begin to address this question in a prospective study on a free-living passerine bird, the tree swallow (*Tachycineta bicolor*), by capitalizing upon naturally-occurring variation in ectoparasitism in 12-day old chicks. We measured body mass, hematological gene expression of the pro-inflammatory cytokine interleukin-6 (IL-6) as well as corticosterone (CORT) secretion at baseline and in response to 30 min of handling. We found that chicks with ectoparasites had smaller body mass and higher levels of IL-6 gene expression at this critical stage of post-natal growth and development. Mass and IL-6 were positively correlated, but only among parasitized chicks, suggesting that larger chicks mount stronger immune responses when necessary, i.e. in the presence of ectoparasites that are known to induce inflammation. IL-6 mRNA expression was negatively correlated with stress-induced CORT levels, suggesting that this proxy of inflammation may be co-regulated with or coordinated by glucocorticoids. More importantly, these endocrine-immune parameters predicted survival to fledging, which was positively associated with IL-6 mRNA abundance and, to a lesser degree, CORT reactivity. These results suggest a link between endocrine-immune interactions and performance in nature, and as a consequence, they shed light on the potentially adaptive, context-dependent interplay between body mass, immunity, and endocrine physiology during development.

1. Introduction

The last decade or so has been marked by a surge of research on the causes and consequences of variation in immune function within and between species, with an increasingly integrated approach that marries the life history perspectives of ecoimmunology with the more mechanism-focused psychoneuroimmunology (Ashley and Demas, 2017; Martin, 2009; Martin et al., 2011; Sheldon and Verhulst, 1996). An emerging theme of this research is the context-dependency of immune function, which is influenced by a number of interrelated extrinsic and intrinsic factors, including endocrine physiology, body condition, and various environmental conditions (Ardia et al., 2010; Demas and Carlton, 2015; Killpack et al., 2015; Lopes, 2014; Martin, 2009; Sheldon and Verhulst, 1996). Comparisons of populations or species occupying different environments mirror these patterns (Ardia, 2007; Martin et al., 2014; Pigeon et al., 2013), suggesting that endocrine-immune interactions may be shaped by natural selection.

Indeed, the ability to appropriately mount an immune response is an integral part of the integrated organismal phenotype (Demas et al., 2011a; Martin et al., 2011). Parasites and pathogens are thought to apply strong selective pressures on hosts, not only to avoid, tolerate, or resist infection, but also to minimize the costs of an immune response and effectively clear infection with limited collateral damage (Ashley et al., 2012; Medzhitov, 2008). The balance between these costs and benefits is shaped by biotic and abiotic environmental conditions, which influence how selection acts on immunity (Hasselquist and Nilsson, 2012; Seppala, 2015). For example, experimental cooling of developing offspring can lead to reduced bactericidal capabilities and lower body condition in birds (Ardia et al., 2010). Likewise, food restriction typically impairs at least some components of the immune system, although these effects vary with the magnitude and type of diet manipulation (Hasselquist and Nilsson, 2012), suggesting a complex

* Corresponding author at: Department of Biology, Indiana University, 1001 E. Third Street, Bloomington, IN 47405, USA.
E-mail address: krosvall@indiana.edu (K.A. Rosvall).
[1] Present address: Department of Biology, Utah State University, Logan, UT 84322, USA.

https://doi.org/10.1016/j.ygcen.2018.08.008
Received 29 March 2018; Received in revised form 18 June 2018; Accepted 4 August 2018
Available online 09 August 2018

interplay between body condition, immunity, and other components of the phenotype. All of these life history and performance traits also interact with parasite or disease status: when animals are experimentally infected during development, they typically experience slower growth and reduced survival (e.g. with blood-sucking fleas, Nilsson, 2003), and vice versa when they are experimentally cleared of infection (e.g. with ectoparasitic nest flies, Koop et al., 2013). Parasites therefore play a critical role linking immune function to other context-dependent intrinsic or extrinsic factors.

The context-dependency between parasites and immune function may be especially strong for the inflammatory response, which is a generalized innate immune response that is among an animal's first line of defense against pathogens, parasites, injury, or other tissue damage (Ashley et al., 2012; Medzhitov, 2008). Inflammation triggers a systemic acute phase response (APR), which involves a suite of behavioral and physiological changes that ultimately aid in survival via a generalized immune response. However, the APR is energetically expensive (Klasing, 2004), and an over-active inflammatory response also may be costly due to the potential for collateral tissue damage (Medzhitov, 2008). Likewise, the APR induces fever, lethargy, anorexia, and other 'sickness' behaviors that may interfere with vital fitness opportunities, such as attracting a mate, defending a territory, or otherwise caring for self or offspring (Ashley et al., 2012; Medzhitov, 2008). Not surprisingly, therefore, inflammatory responses and sickness behaviors can vary from one context to another, including season (Owen-Ashley and Wingfield, 2007), age (Foster et al., 1992; Palacios et al., 2011), and the energetic demands facing an organism, which can be shaped by parental care (Aubert et al., 1997; Bonneaud et al., 2003) or social context (Lopes, 2014).

Several endocrine-immune signaling molecules are likely to coordinate these immune responses, as well as their context-dependency. For example, cytokines are signaling molecules that can act systemically or locally to regulate cellular immunity, inflammatory responses, and related physiological and behavioral changes (Zimmerman et al., 2014). Experimental treatment with lipopolysaccharide, which is a component of the bacterial cell wall, triggers an inflammatory response without an infection and typically elevates specific cytokines in blood (Meitern et al., 2014). Glucocorticoid (GC) hormones are another likely mediator of inflammatory responses, as these steroids are secreted in response to a range of metabolically demanding or otherwise 'stressful' situations. GCs typically facilitate energy mobilization (Sapolsky et al., 2000), which ought to be adaptive during an energetically expensive inflammatory response. Indeed, GCs are known to influence the production of pro-inflammatory cytokines (Borghetti et al., 2009; Dhabhar, 2002; Sapolsky et al., 2000), and they have marked effects on many components of immunity, with some context-dependency. For example, acute stress or short-term exposure to elevated GCs can be immunosupportive and induce leukocyte trafficking from the blood to other parts of the body (e.g., skin, vital organs), whereas chronic, longer-term exposure can be immunosuppressive (Dhabhar, 2009; Dhabhar and McEwen, 1997; Martin, 2009; Sapolsky et al., 2000). Adding further complexity, the links between circulating GCs and immunity may be indirect, mediated instead by their co-regulation via the sympathetic nervous system (e.g. catecholamine signaling), GC receptor expression in immune tissues, or corticotrophin-releasing hormone in the brain, and as well as interactions among these endocrine parameters (Denno et al., 1994; Martin, 2009; Sternberg, 2006).

Here, we sought to understand how endocrine-immune connections relate to performance in nature, by studying nestling tree swallows (*Tachycineta bicolor*). Tree swallows are cavity-nesting insectivorous songbirds that are an emerging model system in ecoimmunology (Ardia, 2007; Ardia and Rice, 2006; Palacios et al., 2009; Palacios et al., 2011; Pigeon et al., 2013). Focusing on 12-day old nestlings, we measured hematological gene expression for the pro-inflammatory cytokine interleukin-6 (IL-6) as a proxy for an individual's inflammatory response. Inflammation affects, and is affected by, hundreds of genes acting systemically and locally (Zimmerman et al., 2014), but we made the *a priori* decision to focus on IL-6 because it is likely to serve as a marker of inflammation. First, IL-6 is one of just three cytokines that are most critical in the systemic pro-inflammatory response, along with TNF-α and IL-1 (Ashley et al., 2012; Medzhitov, 2008). Second, because IL-6 also responds to the production of TNF-α and IL-1 (Zimmerman et al., 2014), its expression is likely to represent an integrated proxy for the pro-inflammatory cytokine response. Furthermore, a number of studies in mammals and birds collectively suggest that patterns of gene expression mirror functional assays of inflammation (Meitern et al., 2014; Owen-Ashley and Wingfield, 2007), and inflammatory responses are deeply conserved over evolutionary time (Zimmerman et al., 2014). Molecular proxies of inflammation also track larger ecological, population, and evolutionary patterns of immunity and parasite exposure (Liebl and Martin, 2013; Martin et al., 2014; Martin et al., 2015); however, it is less clear how *within*-population variation in inflammation-related gene expression relates to other life history parameters, such as survival or growth. Because this among-individual variation is a prerequisite for evolutionary change (e.g. Rosvall et al., 2012a; Whitehead and Crawford, 2006), understanding how endocrine-immune signaling relates to fitness is a key issue in evolutionary endocrinology.

Based on the observation that inflammatory responses are energetically expensive, we predicted that smaller chicks would have lower IL-6 mRNA abundance, and vice versa. Furthermore, we predicted that parasites would influence the relationship between mass and IL-6, which would be strongest when chicks were immunologically challenged. To begin to test this hypothesis, we capitalized upon naturally-occurring variation in parasite status, contrasting nestlings with and without readily visible infections with common ectoparasites (e.g. mites, lice, fleas, see Section 2.1). Ectoparasites have been associated with inflammatory responses in many systems (Braden et al., 2012; Covello et al., 2009; Harrington et al., 2010,b; Koop et al., 2013; Wegmann et al., 2015), providing a foundation for our hypotheses. To assess whether these immune capabilities may be coordinated by, or co-regulated with, glucocorticoid stress hormones, we sampled baseline and handling-induced corticosterone levels in these same individuals. Finally, to begin to understand the fitness correlates of these life history and endocrine-immune interactions, we investigated the degree to which nestling mass, corticosterone, IL-6, and parasite status predicted the likelihood of survival to fledging.

2. Materials and methods

2.1. Field methods

This study occurred in 2015 at artificial nesting cavities ('nestboxes') near Indiana University (39°9 N, 86°31 W). Once territories were established (April), nestboxes were monitored every few days to determine first egg date, hatch date, clutch size, and brood size. Between 27-May-2015 to 25-Jun-2015, we collected data from 12-day-old chicks (n = 152; hereafter "d12") at n = 32 nests (mean ± se for clutch size = 5.6 ± 0.2; brood size = 4.8 ± 1.3, ranging from 2 to 7 chicks/nest). Tree swallows can hatch asynchronously (Winkler et al., 2011), and we denoted hatch day (Day 1) as the date on which the majority of nestlings hatched (Clotfelter et al., 2000), as indicated by damp natal down in the afternoon when the nest was checked. Our focus on d12 chicks is ideal because most chicks reach their asymptotic mass at this age (Quinney et al., 1986), at which point size hierarchies are relatively stable (Ardia, 2006). Mass at this stage is a common proxy of post-natal growth (Gebhardt-Henrich and Richner, 1998), and d12 mass is known to predict post-fledging survival in this species (McCarty, 2001). Furthermore, CORT and many components of innate immunity are on par with adult values by this age (Lynn and Kern, 2014; Lynn et al., 2013; Palacios et al., 2009; Stambaugh et al., 2011).

Upon approaching the nest on d12, we retrieved as many chicks as