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Original investigation

Individual condition and inflammatory response to PHA in the subterranean rodent *Ctenomys talarum* (Talas tuco-tuco): A multivariate approach

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ABSTRACT

Several components of individual condition can modulate immune function in vertebrates. One of the most common techniques used by ecologists to estimate immune responsiveness in wild populations is the phytohemagglutinin (PHA)-skin test, which is usually considered a proxy of cell- mediated activity. To determine the influence of individual condition on the magnitude of this immune index we assessed the variability in the inflammatory response to PHA in the subterranean solitary rodent, *Ctenomys talarum* (Talas tuco-tucos), including 18 variables associated with different aspects of individual condition that are indicative of physical, physiological, parasitological and immunological state. A partial least square (PLS) regression analysis was performed on the condition and the response variables to identify those having greater contribution to overall variation in the response. The first two components explained together 77.5% of the maximum achievable explained variance in the inflammatory response to PHA (60% of total variance). Abundance of eggs of *Trichuris pampeana,* eosinophil, monocyte and neutrophil counts, together with reproductive condition were identified as the most important variables affecting the inflammatory response. The first component negatively associated the inflammatory response to PHA with the abundance of the nematode *T. pampeana*, and with counts of eosinophils and monocytes. The second component negatively associated the inflammatory response to PHA with reproductive condition, neutrophils and eosinophils. We discuss the general negative effect of infection on the immune responsiveness of wild animals and highlight the usefulness of the multivariate approaches in providing information on physiology, parasitology and health relationships to assess patterns of variation in immune responses.

Introduction

Immune function is a critical component of survival for vertebrates (Barnard and Behnke, 2006). However, within species, individuals show notable variation in the magnitude and effectiveness of their immune responses (Schmid-Hempel, 2003). It is known that the individual "condition" –the suborganismal environment in which the immune response manifests itself, defined by competing physiological demands on the host at the time of infection- influences immune performance (Lazzaro and Little, 2009). Several studies have evidenced the effect of different components of individual condition on immune function, but the majority of them have evaluated the influence of only a single or few indices of individual condition over immunity (e.g. genetic factors, Wakelin, 2009; heat and social stress, parental effort, Christe et al., 2011, Powell et al., 2013). Measures of individual condition are not independent indicators of some aspect of organismal functioning, but regulatory components of a single unified physiological system (Cohen et al., 2012). An effort to understand intra- and inter- specific variation in immune responsiveness, by measuring and analyzing a combination of proxies of individual condition, instead of using single parameters or simply body mass-based measures (Jakob et al., 1996; Peig and Green, 2009) is needed to understand the evolution of condition-dependent

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Abbreviations: FEC, fecal egg count; N:L, neutrophil–lymphocyte ratio; NAb, natural antibody; PBS, phosphate buffered solution; PHA, phytohemagglutinin; PLS, partial least squares regression; RBC, red blood cells; SRBC, sheep red blood cells; Th1, type 1T lymphocyte; Th 2, type 2 T lymphocyte.

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immune strategies and to delve deeper into questions of comparative immunology in general (Matson et al., 2006; Pedersen and Babayan, 2011; e.g.: Oliver-Guimerá et al., 2017). Besides, ecological studies of immune variation in wild populations have mostly involved birds, while other groups of vertebrates, such as mammals, remain much less investigated. Particularly, species with unique habits and uncommon life history traits are the focus of major interest for ecoimmunological studies, since they will provide information regarding diverse immune strategies.

The aim of this study was to assess the impact of individual condition on the inflammatory response of *Ctenomys talarum* (Talas tuco-tucos). Individual condition was assessed in an integral fashion, through the estimation of the physical (body weight, hematocrit), physiological (reproductive condition, stress, sex) and immunological/infection (parasite counts, leukocyte counts, agglutination capacity of plasma) state. The inflammatory response was assessed through the phytohemagglutinin (PHA)- skin test. This test has already been characterized in Talas tuco-tucos, which exhibit a large inter-individual variation in the magnitude of the swelling response (Merlo et al., 2014a). Thus, a more comprehensive analysis of the factors potentially associated with this response would contribute to determine the causes of its inter-individual variation. The use of PHA, although controversial regarding the type of immune components stimulated (Kennedy and Nager, 2006), allows the macroscopic quantification of inflammation in a simple manner, excluding additional processes (e.g. tissue damage, complex immune responses) associated with a real pathogenic infection. The Talas tuco-tucos are considered a “slow-living” species; they exhibit low basal metabolic rate (BMR), have long lifespans and long gestation periods (3 months) and give birth to altricial pups, only twice a year (Busch et al., 1989; Zenuto et al., 2002a,b). This contrasts with the majority of rodent species, which exhibit “fast-living” traits. Additionally, Talas tuco-tucos have subterranean habits, living in permanently sealed burrows and restricting most of their activities to these tunnels (Busch et al., 2000). Therefore, *C. talarum* is an interesting species for the study of immune strategies, since their life history traits and habits are uncommon among rodents and mammals in general. Moreover, much is known about the natural history, ecology, and physiology of this species (e.g. Busch et al., 2000; Luna and Antinuchi, 2007; Zenuto et al., 2002a,b), which can contribute to understand the context in which immune function varies. There is a clear synergism between infection and poor condition (Beldomenico et al., 2008a,b), in which parasites have detrimental effects on their host condition through reduction of hostís energy reserves and nutrients (Brown and Brown, 1989; Richner et al., 1993), and, in turn, poor condition predisposes to host infections, given that immunocompetence is impaired. For *C. talarum*, it is known that the energetic cost of the PHA response is low (Merlo et al., 2014a), but nevertheless, a reduction of˜10% of body weight (induced by a low-calorie diet in captivity) reduces the inflammatory response to PHA in a˜ 54% (Merlo et al., 2016a). Also, it has been proposed that parasites trigger the activation of energy-consuming immune mechanisms (Beldomenico et al., 2008a,b; Sheldon and Verhulst, 1996), and can modulate, up-regulating or suppressing, the host resistance to other pathogens (Boughton et al., 2011; Cox, 2001). Based on this, we hypothesized that the inflammatory response of Talas tuco-tucos will be positively associated with a good physical and immunological condition (higher values of body weight, higher hematocrit and innate immunity levels –agglutination capacity of plasma-). In respect with infection, it is known that Talas tuco-tucos have a little diverse parasite fauna, but the prevalence of infection is high, probably as a consequence of burrow conditions that favour transmission (Rossin and Malizia, 2002). A previous study showed that in Talas tuco-tucos, PHA-triggered inflammation is similarly impaired by infection with an intestinal protozoan (*Eimeria* sp.) alone or co-occurring with a number of gastrointestinal nematode species (Merlo et al., 2016b). Thus, in general, we expect a negative association between the inflammatory response and infection (parasite load, leukocyte counts). However, given that immune responses against hematophagous ectoparasites are mainly cell-mediated (Jones et al., 1996; Khokhlova et al., 2004) and that PHA also stimulates this immune branch of the immune system, we expected higher magnitudes of inflammation in response to PHA in individuals with greater loads of fleas, hematophagous mites or lice, as consequence of a potential synergism between immune challenges.

Physiological state of individuals –in this work assessed through the reproductive and stress condition- can also influence the magnitude of immune responsiveness. According to life-history theory, trade-offs between costly activities can maximize fitness and thus, immune suppression appears as a cost of current reproduction (Sheldon and Verhulst, 1996; van der Most et al., 2011), especially during energetically-demanding periods (Ardia, 2005; Deerenberg et al., 1997; Knowles et al., 2009). For this reason, reproductive state of individuals is an important factor to include in our analysis. Even though resource allocation to physiological systems are expected to change seasonally, it was reported not to significantly influence the magnitude of the response to PHA in a previous study in this species (Merlo et al., 2014b), while other studies have reported trade-offs between the inflammatory response to PHA and reproduction (Huyghe et al., 2010; Martin et al., 2004; Møller et al., 2003). Stress is also presumed to affect immunity, and this impact varies according to the duration and intensity of the stressor and the sensitivity of the immune component (Martin, 2009; Sapolsky et al., 2000). Stress is an adaptive response to diverse environmental, social and internal –physiological- stressors (Boonstra et al., 2001), in which energy is mobilized to safeguard immediate survival while other activities and processes like reproduction, growth and immune system are suppressed (see McEwen and Wingfield, 2010 for a review).

Finally, sex differences in immune responses are also well documented in vertebrates (Fargallo et al., 2002; Nunn et al., 2009; Restif and Amos, 2010), where males generally exhibit reduced immune responses as well as increased intensity and prevalence of infections compared to female conspecifics. Although differences in the response to PHA among sexes have not been previously reported for this species (Merlo et al., 2014b), this factor may contribute to the variance in the magnitude of inflammation.

Material and methods

Animal capture and captivity conditions

Adult *Ctenomys talarum* (Talas tuco-tucos) were live-trapped in the locality of Mar de Cobo, Buenos Aires Province, Argentina (37°46′ S 57°27′ W) using wire tube-shaped live traps (10 cm diameter, 35 cm length) set at fresh surface mounds. Nursing females trapped, identified based on their enlarged nipples and missing hair around them, were immediately released back into their burrow system so as not to deprive dependent young of maternal care. Juveniles, which were identified based on their body weight (Malizia and Busch, 1991), were also released. A total of 48 animals were caught. Specifically, 10 males (162 ± 8.61 g) and 10 females (109.16 ± 4.32 g) were caught during the non-reproductive season (April to early May 2012), and 13 males (162.10 ± 4.18 g) and 15 females (122.26 ± 3.43 g) were caught during the reproductive season (August to November 2012). Immediately after capture, feces of each animal were collected from traps (or from the tube in which each individual was transported, up to 5 h after capture) and conserved in 4% formalin. Additionally, a blood smear was