Comparative Biochemistry and Physiology, Part A 162 (2012) 7–15

Contents lists available at SciVerse ScienceDirect

Comparative Biochemistry and Physiology, Part A

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

Baseline haptoglobin concentrations are repeatable and predictive of certain aspects of a subsequent experimentally-induced inflammatory response

Kevin D. Matson *, Nicholas P.C. Horrocks, Maaike A. Versteegh, B. Irene Tieleman

Animal Ecology Group, Center for Ecological and Evolutionary Studies, University of Groningen, P.O. Box 11103, 9700 CC, Groningen, The Netherlands

ARTICLE INFO

Article history:
Received 12 December 2011
Received in revised form 16 January 2012
Accepted 16 January 2012
Available online 31 January 2012

Keywords:
Acute phase proteins
Birds
Circannual patterns
Columbiformes
Ecological immunology
Inflammation
Lipopolysaccharide
Repeatability

ABSTRACT

Ecologists sometimes assume immunological indices reflect fundamental attributes of individuals—an important assumption if an index is to be interpreted in an evolutionary context since among-individual variation drives natural selection. Yet the extent to which individuals vary over different timescales is poorly understood. Haptoglobin, an acute phase protein, is an interesting parameter for studying variability as it is easily quantified and concentrations vary widely due to the molecule's role in inflammation, infection and trauma. We quantified haptoglobin in pigeon plasma samples collected over fourteen months and calculated repeatability to evaluate if haptoglobin concentration is a distinctive trait of individuals. We also explored the capacity of baseline haptoglobin concentrations to predict an array of physiological changes associated with a subsequent experimentally-induced inflammatory response. Maximum repeatability, which occurred over a short mid-winter interval, equaled 0.57. Baseline haptoglobin concentrations predicted response haptoglobin concentrations better than any other endotoxin-induced change. Overall, we identified several strengths and limitations of baseline [Hp] quantification. Acknowledging these qualities should lead to more refined conclusions in studies of the ecology and evolution of immune function.

1. Introduction

Interest in incorporating aspects of immunology into studies of ecology and evolution continues to grow. Indices of immune function used by ecological immunologists are often assumed to reflect fundamental attributes of individuals (Hill, 2011). Such an assumption is important if the measures are interpreted in an evolutionary context since variation among individuals is a driver of natural selection (Hõrak et al., 2002; Tieleman et al., 2010, but see Pitala et al., 2007). The immune system is, however, dynamic. For many immune indices, the extent to which individuals vary over different timescales is poorly understood. Understanding immunological lability requires further investigation over timescales both short (i.e., hourly, daily, or pre- and post-challenge sampling; e.g., Millet et al., 2007; Horrocks et al., 2011)) and long (i.e., monthly or seasonally sampling over one or more years; e.g., Hõrak et al., 2002; Buehler et al., 2008, Hegemann unpublished data).

Haptoglobin (Hp), in particular, and acute phase proteins, more generally, are especially interesting parameters for studying immunological lability. First and foremost, concentrations within individuals vary through time and mirror changes in health status and physiological condition (Hõrak et al., 2002, 2003). Under normal conditions (e.g., in the absence of acute pathologies), Hp circulates in the blood at low, but taxon-specific, concentrations. Concentrations can increase rapidly and manifoldly in response to acute infection, inflammation, or trauma (Delers et al., 1988; Millet et al., 2007). An elevated plasma haptoglobin concentration ([Hp]) often signifies the onset of a non-specific immune response, but changes might also reflect the role of Hp as an antioxidant (Gutteridge, 1987; Dobryszycka, 1997; Quaye, 2008).

Hp and its functional equivalents have been identified, either genetically or functionally, in a wide range of taxa (Delers et al., 1988; Matson, 2006; Matson et al., 2006a; Wicher and Fries, 2006; Millet et al., 2007; Kakuschke et al., 2010). Mean [Hp] varies among bird species (Delers et al., 1988; Matson, 2006; Matson et al., 2006a; Millet et al., 2007), but the highest concentrations reported in birds are generally lower than the highest concentrations reported in mammals. The use of a simple, commercially-available, functional assay means that plasma [Hp] can be easily quantified in the wide variety of avian species that is often encountered with comparative ecological approaches (Matson, 2006; Matson et al., 2006a; Millet et al., 2007). Nevertheless, a firm grip on how to interpret [Hp] in the context of these comparative studies has remained elusive (e.g., Matson, 2006).

The connections between health status, physiological condition, and immune function embody one source of variability in many indices of immune function, including Hp. While such connections are central to some questions in ecological immunology, these very same connections can complicate interpretations of the causes and consequences of any observed differences (Hill, 2011). For example,

* Corresponding author. Tel.: +31 50 3632181; fax: +31 50 3635205.
E-mail address: k.d.matson@rug.nl (K.D. Matson).

1095-6433/$ – see front matter © 2012 Elsevier Inc. All rights reserved.
doi:10.1016/j.cbpa.2012.01.010

the quantification of [Hp] in the context of comparative ecological studies often permits only equivocal and unsatisfying conclusions about costs and trade-offs (Matson, 2006; Buehler et al., 2009b), even with the molecule's unambiguous association with energetically-expensive systemic inflammation (Buehler et al., 2009a; van de Crommenacker et al., 2010). This lack of clarity results from gaps in the basic ecological understanding of acute phase proteins, such as the extent to which constitutive [Hp] predicts the magnitude of an acute phase response. Field studies where individuals are observed only at a single point in time (i.e., the point of capture and sampling) further complicate interpretation, and in these cases the degree to which plasma [Hp] is an attribute of individuals cannot be determined. More information might be known at the level of population (e.g., life history stage, habitat quality) or species (e.g., susceptibility to particular disease, geographic range) in field-studies, but these ecological correlates alone are unlikely to reveal the causes of differences in average [Hp] among groups (Matson, 2006; Buehler et al., 2009b).

To improve upon the current ecological understanding of avian Hp, we examined the variability in "baseline" or constitutive (i.e., non-experimentally-induced) [Hp] in the contexts of repeatability and predictive capacity. Repeatability, which is defined as the portion of total variation that is attributable to among-individual differences (Lessells and Boag, 1987), signals the degree to which baseline [Hp] is a distinctive trait of individuals. We defined predictive capacity as the strength of a relationship between a baseline physiological measurement (in our case, [Hp]) and a subsequent physiological change that results from an immunological challenge. We used this concept of predictive capacity to help identify potential consequences of among individual differences.

We measured baseline [Hp] in 13 plasma samples collected repeatedly from 16 homing pigeons over the course of 14 months. We followed this baseline sampling scheme with an experiment designed to assess the physiological effects of an endotoxin-induced acute phase response. First, we investigated repeatability within intervals that comprised multiple consecutive samples but that differed in length (between two and thirteen approximately-monthly measurements) and time of year. We asked two questions: 1) to what extent is [Hp] a repeatable and distinctive trait of individuals? and 2) to what extent is this repeatability influenced by the time of year and the number of repeated measurements in a particular series of samples? We also tested the effects of sex and sample month on repeatability in specific sample series. Second, we explored the correlations between the within-individual means of baseline [Hp] and the within-individual changes in physiological parameters following an experimental immunological challenge. We asked the following question: within individuals, to what extent is baseline [Hp] predictive of the effects of an endotoxin-induced inflammatory response? For this purpose, we quantified changes in an array of physiological and immunological indices that are frequently used by ecologists: body mass, cloacal temperature, metabolic rate, oxidative status, blood glucose concentration, hemolysis, hemagglutination, bacteria killing abilities, and [Hp]. As with repeatability, we investigated if the predictive capacity of baseline [Hp] is influenced by the time of year and the number of repeated measurements used to calculate the predictor.

2. Materials and methods

2.1. Birds

We studied 16 homing pigeons (eight ♀, eight ♂; *Columba livia domestica*). Birds lived year-round in single sex groups of four in small outdoor aviaries (roughly 2 m × 3 m × 2 m high). Birds accessed food (a mix of seed and pellet diets, grit, and vitamins) and water ad libitum. Hatched between late November and late December in 2005, all birds were about one year and five months old at the initiation of sampling and about two years and eight months old at the time of the endotoxin challenge (August 2008). For additional information about the study system, see van de Crommenacker et al. (2010).

2.2. Blood sampling, endotoxin challenge, and metabolic measurement

We used heparinized syringes to collect blood samples (<1 mL, <0.5% of body mass) from all birds by brachial venipuncture. We centrifuged the whole blood and collected and froze (−20 °C) the plasma fraction for future analyses. Approximately two to three hours elapsed between collection and freezing; blood samples were kept cool (~4 °C) during this period.

We collected 13 baseline plasma samples from each bird at approximately-monthly intervals between April 2007 and June 2008 (mean interval = 36 days ± 11 days (s.d.)). On each baseline sample day, we collected blood from all birds over the course of the afternoon (between ~13:00 and ~18:00 h). Among cages, we followed a rotating sampling order with different starting cages on different baseline sample days; within cages, we caught and sampled birds in random order.

In August 2008 we collected pre- and post-endotoxin-challenge samples from eight birds (four ♀, four ♂). Pre- and post-endotoxin-challenge samples were always separated by a 48-hour interval. Both blood samples were collected from birds between 11:00 and 11:30, which was after 22 h of fasting and 18 h in a metabolic chamber at thermoneutral conditions. We administered the endotoxin challenge between 17:00 and 17:30, which was immediately before the birds' second entry into the metabolic chamber and 18 h before the collection of post-challenge samples. The endotoxin challenge was an intraperitoneal injection of 2 mL per kg body mass of a 1.25 mg mL^{-1} solution of lipopolysaccharide (LPS, L7261; Sigma, MO, USA) dissolved in phosphate buffered saline.

During the two 18 h periods each bird spent in a metabolic chamber, we used standard flow-through respirometry to measure oxygen consumption, which we divided by average nightly mass ($mL\ h^{-1} g^{-1}_{body\ mass}$) (Gessaman, 1987). Because the respirometer was set up to measure two birds per night, the experimental protocol was staggered in time over a two-week period. For complete details about the metabolic set-up and equipment, see van de Crommenacker et al. (2010). This experiment was approved by the Animal Experimentation Committee of the University of Groningen (DEC license number 5095).

2.3. Haptoglobin quantification: baseline and endotoxin-challenge experiment

We quantified [Hp] (mg mL^{-1}) in all baseline blood samples and in pre- and post- challenge blood samples. We used a commercially-available functional assay (TP801; Tri-Delta Diagnostics, NJ, USA), which colorimetrically quantifies the heme-binding capacity of plasma. We followed the 'manual method' instructions provided by the kit manufacturer with a few minor modifications. Because preliminary measurements revealed low [Hp] in pigeons, we used twice the standard amount of plasma per well (15 μL instead of 7.5 μL) and adjusted the calculated concentrations accordingly. We measured absorbances at two wavelengths (450 and 630 nm) prior to the addition of the final reagent that initiated the color-change reaction. The pre-scan at the normal assay wavelength of 630 nm allowed us to correct for differences in plasma color and cloudiness by subtracting pre-scan absorbance values from final absorbance values. The 450 nm pre-scan enabled us to statistically analyze and correct for differences in plasma sample redness, an indication of hemolysis, which can affect the assay (see appendix).

Based on an among-plate standard that was run in duplicate in each of the five assay plates used in this study, within-plate variation averaged 3.7%, and between-plate variation equaled 3.5%. Based on 32 pigeon plasma samples that were run in duplicate in a single plate, within-plate variation averaged 5.0%.