Bat immune systems may allow them to respond to zoonotic agents more efficiently than other mammals. As the first line of defence, the taxonomically conserved acute phase immune reaction of leucocytosis and fever is crucial for coping with infections, but it is unknown if this response is a key constituent to bat immunological success. We investigated the acute phase reaction to a standard lipopolysaccharide (LPS) challenge in Pallas’s mastiff bats (Molossus molossus). Challenged bats lost mass, but in contrast to other mammals showed no leucocytosis or fever. There also was no influence on body temperature reduction during torpor. When compared to recent genome-wide assays for constituent immune genes, this lack of a conserved fever response to LPS contributes to a clearer understanding of the innate immune system in bat species and of the coevolution of bats with a wide diversity of pathogens.

1. Introduction

Bats host more zoonotic virus species than other mammalian orders, largely without presenting incidence of disease [1,2]. This renders bats potential reservoir hosts and relevant research has focused on specific antibody responses to pathogens [1–3]. The less-investigated first line of defence of the immune response, the acute phase reaction, contributes to the early control of infections and may have influenced bat coevolution with a wide diversity of pathogens that are highly virulent in other species. What little is known about bat acute phase response suggests that they are no different than other mammals [1,4] although there may be novel adaptations to successfully cope with infections [3,4].

Increases in body temperature ($T_B$, fever), circulating white blood cells (WBC, leucocytosis) and mass loss are common parts of the conserved acute phase reaction and can be induced experimentally [5–7]. Fever is especially important in combating infections by inhibiting viral and bacterial replication as well as enhancing leucocyte responses to infection [6]. This metabolic and temperature cycling occurs in bats on a daily basis as they transition from resting states to $T_B$ greater than 40°C during flight, which is hypothesized to mimic daily fever [3]. At the other end of the spectrum, many bats lower $T_B$ and metabolic rate on a daily basis through torpor and seasonally through hibernation. Such metabolic suppression can have broad effects on immune function. For example, during hibernation, fever response is delayed in ground squirrels until they return to normothermic temperatures [8], and immune challenges prevent full suppression of $T_B$ during torpor in grey mouse lemurs [9]. To test the acute phase responses in Pallas’s mastiff bats (Molossus molossus), we used a standard acute phase immune challenge.
designed to elicit both fever and leucocytosis. _Molossus molossus_ use torpor as a daily energy-saving strategy [10], and we hypothesized that if bat acute phase immune reactions are concordant with mammalian patterns, lipopolysaccharide (LPS) injection should reduce daily temperature fluctuations [9], and/or that daily return to normothermy should trigger a delayed fever response [8]. Furthermore, we hypothesized that LPS should induce the standard responses of mass loss and leucocytosis.

### 2. Material and methods

We captured bats at evening emergence from roosts in Gamboa, Panama (9°07' N, 79°42' W; 2008–2014). We recorded sex, reproductive status, mass and age from all individuals and produced blood smears from a drop of blood collected in a heparin-coated capillary tube [11]. Most individuals (_n_ = 60) were then released within 2 h of capture. We kept six females and seven males for experiments and housed them singly in boxes (20 × 15 × 6 cm) at ambient temperature (±28 °C) and light. They received _ad libitum_ water and were hand fed each evening with _ca_ 2 g of mealworms and cat food (Whiskas, Germany). Subcutaneous thermo-sensitive PIT tags (0.1 mg) were implanted at the beginning of the experiment. Females in this group received the second treatment after 26–28 h post-injection. Females in our experimental set-up and the WBC response was consistent with previous work [13]. Electronic supplementary material, tables S1 and S2, provide all data and counts from individual bats.

**3. Results**

The lack of leucocytosis in _M. molossus_ is unusual. Leucocytosis is a generalized response in vertebrates, and we confirmed the potency of our reagents in a different bat species (electronic supplementary material, tables S1 and S2, figure S1). Baseline circulating WBC in _M. molossus_ were low, even compared to similarly sized bat species (three to four WBC per visual field at 200 × [11]). Low _T_B during seasonal hibernation reduces the number of circulating leucocytes in other mammals [19], but _M. molossus_ reduces...
Initial blood samples were collected from bats caught at evening emergence and held for at least 30 min before sampling. This stress should increase reactivity to LPS [20] if these bats were to mount a response. Further exploration is needed, particularly in bat species that use a broader range of $T_B$ than that possible in the tropics.

The extent to which bat immune systems differ from other mammals remains unclear. So far, hypotheses that link bat immunology to the evolution of flight best explain genome-level immune adaptations. This includes mitochondrial adaptations to metabolic stress and reduction of free radicals [1], increased metabolic rates and body temperatures [3], and trade-offs with immune gene families [4]. Direct tests of the constituent immune responses, however, are necessary to identify the unusual ways in which these adaptations are truly employed [1,3,4]. Our results show that the conserved immune response of fever may no longer be intact [6], which would not be predicted from genomes that indicate intact response pathways [4]. We are beginning to understand the complexity of bat adaptations to infection from genome to basic responses, but bat immunology is still in its infancy.

**Ethics.** Experiments were approved by ANAM, Panama (SE/A-112-13; SE/A-73-14) and the IACUC of the Smithsonian Tropical Research Institute (2012-0905-2015; 2014-0701-2017).

**Data accessibility.** The dataset supporting this article has been uploaded as part of the electronic supplementary material.

**Authors’ contributions.** S.S. and M.T.O. carried out the experiments, statistical analysis and wrote the manuscript. D.K.N.D. and R.A.P. helped design the study and wrote the manuscript. All authors gave final approval for publication.

**Competing interests.** The authors have no competing interests.

**Funding.** This work was funded by the Max Planck Institute for Ornithology, Smithsonian Tropical Research Institute, and German Research Foundation (DFG) award DE 1807/3-1 to D.K.N.D.

**Acknowledgements.** We thank C. Voigt for suggestions with experimental design, the Gamboa BatLab for help in the field and the IMPRS for Organismal Biology writing course, as well as three anonymous reviewers for helpful comments.

**References**


