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Diurnal rhythms in peripheral blood immune cell numbers of domestic pigs

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ABSTRACT

Diurnal rhythms within the immune system are considered important for immune competence. Until now, they were mostly studied in humans and rodents. However, as the domestic pig is regarded as suitable animal model and due to its importance in agriculture, this study aimed to characterize diurnal rhythmicity in porcine circulating leukocyte numbers. Eighteen pigs were studied over periods of up to 50 h. Cosinor analyses revealed diurnal rhythms in cell numbers of most investigated immune cell populations in blood. Whereas T cell, dendritic cell, and eosinophil counts peaked during nighttime, NK cell and neutrophil counts peaked during daytime. Relative amplitudes of cell numbers in blood differed in T helper cell subtypes with distinctive differentiation states. Mixed model analyses revealed that plasma cortisol concentration was negatively associated with cell numbers of most leukocyte types, except for NK cells and neutrophils. The observed rhythms mainly resemble those found in humans and rodents.

1. Introduction

Diurnal rhythms represent adaptations of living organisms to the alteration between day and night and are ubiquitous in the living world (Bell-Pedersen et al., 2005). They mainly result from synchronization to light or darkness (Challet, 2007) but can also be influenced by other external stimuli, called zeitgebers, such as feeding time (Patton and Mistlberger, 2013). Daily rhythmic variations can be found in many behavioral and physiological functions (Panda, 2016; Saper et al., 2005) as well as in the immune system (Carter et al., 2016; Cermakian et al., 2013; Curtis et al., 2014; Geiger et al., 2015; Labrecque and Cermakian, 2015). In this respect, diurnal variations in the cell numbers of different leukocyte subtypes in peripheral blood can be found (Ackermann et al., 2012; Born et al., 1997; Haus et al., 1983; Lasselin et al., 2015; Scheiermann et al., 2012). They seem to result from diurnal regulation of hematopoiesis and leukocyte trafficking between blood, the marginal pool, and different tissue sites (Besedovsky et al., 2014b; Druzd et al., 2014; Méndez-Ferrer et al., 2009; Scheiermann et al., 2012; Suzuki et al., 2016). All of these processes seem to be mediated by main endocrine mediators, such as glucocorticoids and catecholamines (Besedovsky et al., 2014b; Webster et al., 2002), adrenergic innervation into different tissues (Scheiermann et al., 2012; Suzuki et al., 2016), as well as to involve the master circadian clock in the *suprachiasmatic nucleus* of the brain (Curtis et al., 2014) and peripheral clocks in immune cells (Bollinger et al., 2011; Nguyen et al., 2013). In general, diurnal rhythms in the immune system are thought to assure temporally adjusted reactivity and maintenance in immune defense (Cermakian et al., 2013). Chronic disruption of diurnal rhythmicity is associated with a dysregulation of the immune system (Castanon-Cervantes et al., 2010) and possibly with an increased risk for different diseases (Cermakian et al., 2013). Thus, diurnal immune rhythms potentially influence the onset and progression of infections or diseases (Bechtold et al., 2010; Edgar et al., 2016) and might also affect the outcome of therapeutic interventions like, e.g., vaccination or medication (Long et al., 2016; Smolensky and Peppas, 2007).

Until now, diurnal rhythms in the immune system were mainly studied in humans and nocturnal rodents (Scheiermann et al., 2013). Other model systems are rare in this field of science and for the domestic pig (*Sus scrofa domestica*) there are no studies showing diurnal rhythms in the cell numbers of various leukocyte subtypes so far. However, due to its high anatomical similarity to humans (Renner et al., 2016) and a genome sequence, which is more similar to humans than that of mice (Wernersson et al.,

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2005), the domestic pig is already regarded as highly suitable animal model (Aigner et al., 2010; Gerdts et al., 2015; Meurens et al., 2012). Moreover, the domestic pig is diurnally active like humans (Ingram et al., 1980; Ingram and Dauncey, 1985) and its high blood volume enables intraindividual blood collection, which altogether predisposes the domestic pig as valuable model for the analysis of diurnal immune rhythms and their underlying mechanisms as well as their influence on immune responses. In addition, from a practical point of view, high immune competence is important to prevent infectious diseases in pig stocks and to improve animal welfare (Colditz, 2002). The knowledge of factors potentially influencing this competence is therefore of high relevance for management in pig husbandry systems and veterinary interventions.

For these reasons, the aim of the present study was the characterization of diurnal rhythms in peripheral blood immune cell numbers in domestic pigs held under light-entrained conditions with concentrate feeding two times a day. This mimics usual practices in animal husbandry as well as life-habits of humans. We evaluated diurnal rhythmicity in immune cell numbers as well as activity behavior, plasma cortisol concentration, and hematocrit with cosinor analysis (Nelson et al., 1979). In addition, linear mixed model analysis was performed to assess potential associations between the investigated parameters.

2. Materials and methods

2.1. Animals and surgery

All procedures were conducted in accordance with the German Animal Welfare Act and approved by the local Animal Welfare Ethics Committee (Regional Council Stuttgart, approval number V309/13TH). A total of 18 castrated male pigs (Piétrain × German landrace, 6-month-old, body weight (BW) range 92–106 kg) were included in the study. The pigs were housed in a lightproof building of the experimental unit of the department Behavioral Physiology of Livestock at a constant ambient temperature of 21 ± 1 °C. Animals were kept in individual pens (6.4 m^2 each) with sight and tactile contact to neighboring animals and had *ad libitum* access to hay and water. They were fed concentrate (1.2 kg/meal, ME 12 MJ/kg) twice daily at 07:30 h and 15:30 h. Pens were cleaned and littered with dust-free wood shavings daily after concentrate feeding in the morning. All pigs were maintained under a 12:12 light-dark cycle (lights on 07:00 h to 19:00 h) with on average 190 lx at pigs' eye level during the light phase (fluorescent tubes, Philips Master TL-D Super 80 58W/840, color temperature 4000 K) and 0 lx during the dark phase. All pigs were accustomed to the lighting and feeding regime for at least 8 weeks prior to the experiments and well habituated to human handling. To obtain blood samples without disturbing the animals, all pigs were surgically cannulated with indwelling vein catheters (*vena cava cranialis*) at least 2 weeks prior to sampling. The surgical procedure was conducted according to Kraetzl and Weiler (1998) with modifications. Anesthesia was introduced with a combination of ketaminhydrochloride (15 mg/kg BW intramuscular (i.m.), Ursotamin, Serumwerk Bernburg, Bernburg, Germany) and azaperone (2 mg/kg BW i.m., Stresnil, Sanochemia Pharmazeutika, Neufeld/Leitha, Austria) in all animals and general anesthesia was maintained with isoflurane inhalation (1.5–2.5%, Vetflurane, Virbac, Carros, France). Meloxicam (0.4 mg/kg BW i.m., Emdocam, Emdoka, Hoogstraten, Belgium) was used as analgesic. Catheters were exteriorized in the neck area and stored in a pouch fixed on the skin. Catheters were rinsed with heparinized saline (115 IU/ml, heparin sodium salt, Carl Roth, Karlsruhe, Germany) twice daily after concentrate feeding. All animals were weighed once per week (not during sampling periods) and health was monitored by daily measurement of rectal temperature.

2.2. Experimental protocol and sample processing

The study was subdivided into three different experimental trials (n = 6 each), which were conducted in spring 2014 and spring 2015 (Fig. 1). Blood sampling started at 10:00 h and was repeated every 2 h in all trials. In the first two trials a total of 12 blood samples were taken until 08:00 h the following day (duration 22 h each). The third trial included a total of 26 blood samples and sampling ended at 12:00 h on the second following day (duration 50 h). Blood sampling at night was performed under dim light of averagely 7 lx at pigs' eye level, which was switched on and off for sampling (Philips energy-saving/LED bulbs 3W, color temperature 2700 K). Sampling all animals lasted not longer than 20 min in total per sampling. Animals were sampled in the same order each time. After discarding the heparinized saline solution from the catheters, 10 ml blood per sample was drawn. Subsequently the catheter was rinsed with approximately 10 ml heparinized saline (46 IU/ml) to keep the catheter patent and to compensate for the blood volume taken. Blood was transferred directly into lithium heparin tubes and K3 EDTA tubes (both Sarstedt, Nümbrecht, Germany). Blood samples were immediately processed after each sampling.

2.3. Hematology

To obtain total leukocyte counts and hematocrit, hematology measurements of K3 EDTA blood samples were carried out using an automated hematology analyzer (MEK-6108G, Nihon Kohden, Rosbach, Germany). All samples were measured in duplicate. The intra-assay coefficient of variation for biological samples was 1.0% for leukocyte counts and 0.7% for hematocrit. Hematology analyses were finished within 60 min after blood sampling.

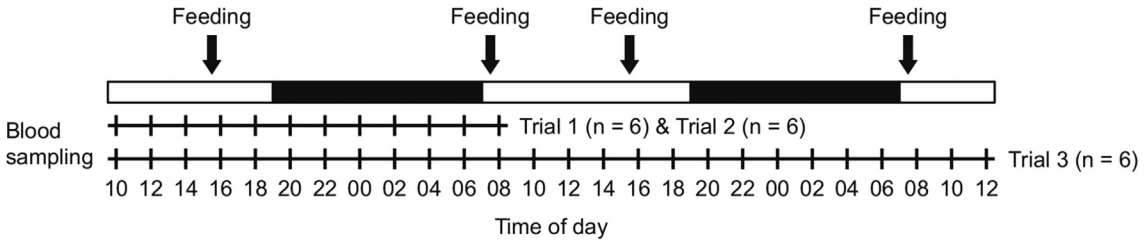

Fig. 1. Experimental protocol. White bars indicate light periods, black bars indicate dark periods, and arrows indicate concentrate feeding times. A total of 18 pigs were studied in three different experimental trials (n = 6 each) under identical experimental settings of 12:12 light-dark cycles. Blood sampling started at 10:00 h and was repeated every 2 h. Twelve pigs were sampled for a total duration of 22 h and 6 pigs were sampled for a total duration of 50 h.