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Acute phase response to *Mycoplasma haemofelis* and '*Candidatus* Mycoplasma haemominutum' infection in FIV-infected and non-FIV-infected cats

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ABSTRACT

The pathogenicity of Haemoplasma spp. in cats varies with '*Candidatus* Mycoplasma haemominutum' (CMhm) causing subclinical infection while *Mycoplasma haemofelis* (Mhf) often induces haemolytic anaemia. The aims of this study were to characterise the acute phase response (APR) of the cat to experimental infection with Mhf or CMhm, and to determine whether chronic feline immunodeficiency virus (FIV) infection influences this response. The acute phase proteins serum amyloid A (SAA), haptoglobin (Hp) and α-1-acid glycoprotein (AGP) concentrations were measured pre-infection and every 7–14 days up to day 100 post-infection (pi) in cats infected with either Mhf or CMhm. Half of each group of cats (6/12) were chronically and subclinically infected with FIV. Marbofloxacin treatment was given on days 16–44 pi to half of the Mhf-infected cats, and on days 49–77 pi to half of the CMhm-infected cats.

FIV-infected animals had significantly lower AGP concentrations, and significantly greater Hp concentrations than non-FIV-infected cats when infected with CMhm and Mhf, respectively. Both CMhm and Mhf infection were associated with significant increases in SAA concentrations, while AGP concentrations were only significantly increased by Mhf infection. Mhf-infected cats had significantly greater SAA concentrations than CMhm-infected animals. Both Mhf and CMhm infections were associated with an APR, with Mhf infection inducing a greater response. Chronic FIV infection appeared to modify the APR, which varied with the infecting Haemoplasma species.

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Introduction

Feline haemoplasmas are haemotropic mycoplasmal bacteria. *Mycoplasma haemofelis* (Mhf) is the most pathogenic, often inducing haemolytic anaemia in immunocompetent cats, whilst '*Candidatus* Mycoplasma haemominutum' (CMhm) and '*Candidatus* Mycoplasma turicensis' do not usually cause anaemia unless concurrent disease or immunosuppression is present (Tasker et al., 2009; Tasker, 2010). Subclinical carrier states also exist and the presence of infection does not confirm that haemoplasmosis is the cause of the clinical signs (Tasker, 2010).

The acute phase response (APR) is part of the innate immune response, characterised by changes in serum acute phase protein (APP) concentrations (Cerón et al., 2005), which can be used as diagnostic and therapeutic biomarkers (Cerón et al., 2005; Griebsch et al., 2009; Mitchell et al., 2009). Measurement of APPs is widely used in human medicine (Berbari et al., 2010; Patel et al., 2010; Sage et al., 2010), and there is increasing interest in their use in companion animals (Eckersall, 2010), although there have to date been limited studies carried out in cats (Harvey and Gaskin, 1978; Duthie et al., 1997; Kajikawa et al., 1999; Sasaki et al., 2003; Paltrinieri et al., 2007a,b, 2008; Tamamoto et al., 2008, 2009). To the authors' knowledge, the only study evaluating the APR to haemoplasmosis in cats reported elevated haptoglobin (Hp) concentrations in six animals experimentally infected with Mhf (Harvey and Gaskin, 1978).

The aim of the present study was to characterise the APR to experimental infection with Mhf or CMhm by measuring serum concentrations of three APPs, serum amyloid A (SAA), haptoglobin (Hp) and α-1-acid glycoprotein (AGP). The influence of chronic feline immunodeficiency virus (FIV) infection on the APR to haemoplasma infection was also assessed.

Materials and methods

Study design

This study was performed on serum samples obtained from previous research (Tasker et al., 2006a,b; Fig. 1). All procedures and experiments described were undertaken under a project license approved under the UK Animals (Scientific Procedures) Act 1986. Briefly, these studies had used 24 specific pathogen free (SPF)-derived cats from one of four groups (n = 6 in each case): Groups A and B were

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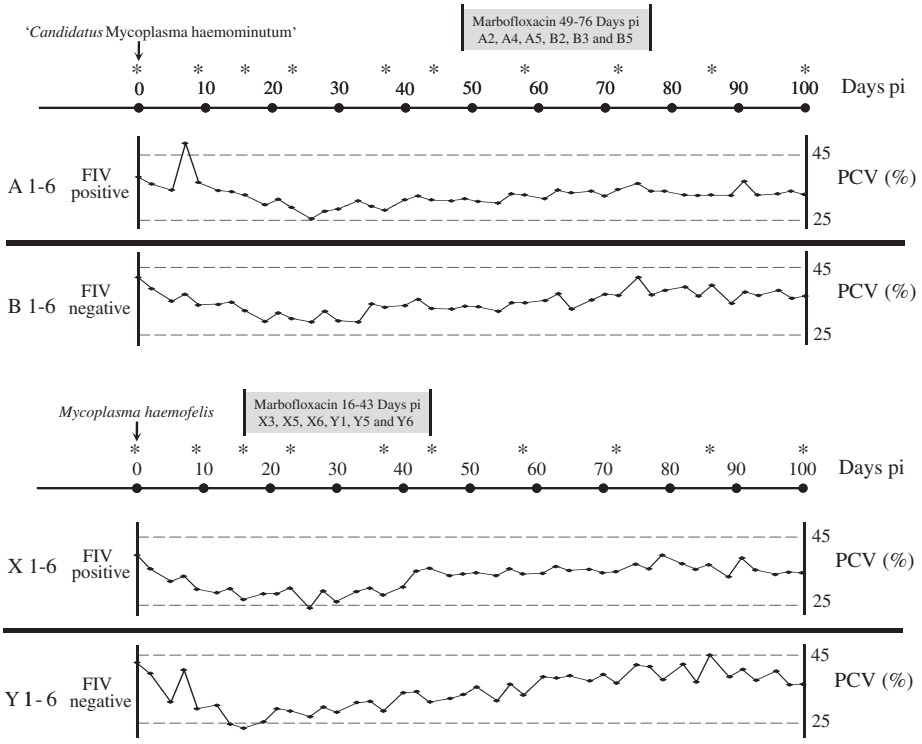

Fig. 1. Schematic illustration of the experimental design for Groups A, B, X and Y. Protocols are indicated against days post-infection (pi). FIV infection status and mean packed cell volume (PCV) (dashed lines indicate reference interval) for each group are indicated. Solid arrow, IV inoculation with Haemoplasma spp.; *sampling times; shaded box, marbofloxacin administration (identity of treated cats shown).

infected with CMhm and Groups X and Y with Mhf, by administering 2 mL of heparinised blood IV from a CMhm- or Mhf-infected donor cat. Groups A and X were chronically infected (23–32 months) with FIV (Glasgow8 strain).

All cats were clinically normal at study commencement. Three cats from each group were randomly selected and given marbofloxacin (Marbocyl, Vétoquinol) at 2 mg/kg orally, once daily for 28 days: between days 49–76 post-infection (pi) for the CMhm-infected cats (cat numbers A2, A4, A5, B2, B3 and B5), and between days 16–43 pi for the Mhf-infected cats (cat numbers X3, X5, X6, Y1, Y5 and Y6) (Tasker et al., 2006a,b). Differences in the timing of this treatment reflected the different time courses of infection: the Mhf-infected cats were treated early in the course of infection when anaemia was present, while the CMhm-infected cats, with subclinical infection, were treated later in the course of infection when CMhm copy numbers remained high.

Each group received the marbofloxacin treatment in order to evaluate the response of each Haemoplasma spp. All cats were given subcutaneous fluid therapy (lactated Ringer's solution) if their PCV fell below 15% and/or the animal exhibited evidence of dehydration. Serum samples were collected pre-infection (day 0), weekly between days 9–44 pi (excluding day 30 pi), and then fortnightly until day 100 pi.

Assays for acute phase proteins

Serum amyloid A concentrations were determined using an immunoturbidimetric method used in humans and validated in cats (Hansen et al., 2006). Alpha-1-acid glycoprotein concentrations were measured using a rapid immunoturbidimetric assay validated for use in cats (Bence et al., 2005). Haptoglobin concentrations were determined using a commercially available kit (Tridelta Development Ltd.). The SAA concentration was measured first, followed by the AGP and Hp concentrations, although inadequate amounts of serum were available to analyse all APPs in all samples: in Group A, seven samples were not analysed for Hp and three were not analysed for AGP; in Group B, four samples were not assessed for Hp or AGP; in Group X, five and two samples were not analysed for Hp and AGP, respectively; and in Group Y, one sample not examined for Hp or AGP.

Statistical analysis

Data were entered into and validated within a database (Excel 2008, Microsoft), and then exported into SPSS software (version 18.0) for further analysis. Significance was set at $P < 0.05$. Statistical analysis was only performed on APP concentrations measured pre-treatment to ensure no confounding effects of treatment. To assess the effect of chronic FIV infection on the APR to haemoplasma infection, Mann–Whitney *U* tests were used to compare each APP concentration pi between the FIV-infected and non-FIV-infected animals, for both CMhm and Mhf. To assess the effect of each infection on the APR, the mean of the APP concentration pi (on days 9, 16, 23, 37 and 44 for CMhm and on days 9 and 16 for Mhf, respectively) was compared with pre-infection (day 0) values using a Wilcoxon-signed ranks test. Conventional two-tailed testing was used to assess changes in Hp and AGP concentrations.

As SAA pre-infection concentrations were below detection limits, the SAA concentration changes pi were investigated using one-tailed testing. These analyses were carried out using non-parametric tests due to the small sample sizes. Two-way repeated measures ANOVA were performed for each APP on days 9–16 pi (the pre-treatment measurements available for both CMhm and Mhf), using FIV and Haemoplasma spp. as grouping variables. Residuals from this analysis tested satisfactorily for normality and homogeneity of variance, confirming the suitability of this test.

Results

Effect of chronic FIV infection on the acute phase response to haemoplasma infection

Pre-existing FIV infection did not significantly affect SAA concentrations following CMhm or Mhf infection, Hp concentrations following CMhm infection, or AGP concentrations following Mhf infection (Table 1). Therefore, the SAA concentration data for CMhm-infected (Groups A and B) and Mhf-infected (Groups X and Y) cats, the Hp data for CMhm-infected (Groups A and B) cats, and the AGP data for Mhf-infected (Groups X and Y) cats, were combined for further analyses.

FIV infection did however affect Hp concentrations following Mhf infection, with FIV-infected cats having significantly greater Hp concentrations than non-FIV-infected animals (Table 1). FIV infection also affected AGP concentrations following CMhm infection, with FIV-infected cats having significantly lower AGP concentrations than non-FIV-infected animals (Table 1). Thus, Hp concentrations in Mhf-infected cats (Groups X and Y), and AGP concentrations in CMhm-infected cats (Groups A and B), were analysed separately.

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