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Caprine herpesvirus-1-specific IgG subclasses in naturally and experimentally infected goats

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ABSTRACT

Enzyme-linked immunosorbent assays (ELISAs) were developed to detect caprine herpervirus-1 (CpHV-1)-specific IgG1 and IgG2 in sera from 43 naturally infected goats. The analysis of the IgG subclasses showed a dual pattern of distribution in seropositive goats with a major group of animals (36 out of 43) exhibiting significantly higher levels of IgG2 over IgG1 and a minor group (7 out of 43) possessing equal levels of IgG1 and IgG2. Four goats were experimentally infected with a virulent CpHV-1 Ba.1 strain by the intranasal or the intravaginal route and the kinetics of appearance of CpHV-1-specific IgG, IgG1 and IgG2 in the serum were studied. Two weeks following infection, both IgG1 and IgG2 levels increased although convalescent sera (i.e., collected 5–8 weeks post-infection) showed a clear prevalence of the IgG2 subclass. To determine the contribution of the different IgG subclasses to herpesvirus immunity, serum neutralization (SN) assays were performed in both naturally and experimentally infected goats. The kinetics of SN showed that neutralization activity was mainly associated to the IgG1 subclass and this was also confirmed in naturally infected goats. The results are discussed from the standpoint that the profile of the IgG subclasses is instrumental to study immune responses to CpHV-1 and that vaccination strategies may benefit from this information.

1. Introduction

Caprine herpesvirus-1 (CpHV-1) belongs to the *Herpesvirales* order, *Herpesviridae* family, *Alphaherpesvirinae* subfamily, *Varicellovirus* genus (Davison et al., 2009) and causes two different syndromes depending on the age of the animal at the time of infection. In adult goats, the infection is often subclinical or it results in vulvovaginitis, balanoposthitis or spontaneous abortion (Grewal and Wells, 1986; Saito et al., 1974; Tarigan et al., 1987; Williams et al., 1997). In 1- or 2-week old kids, CpHV-1 is responsible for a systemic disease characterized by high morbidity and mortality, and ulcerative and necrotic lesions throughout the enteric tract (Mettler et al., 1979; Van der Lugt and Randles, 1993). CpHV-1 enters the animals via the genital or the respiratory tract and establishes latent infection in the sacral and trigeminal ganglia (Tempesta et al., 1999b). In natural infection, it can reactivate during the mating season probably as a result of the stress associated to hormonal changes (Tempesta et al., 1998) while in experimental infection, reactivation can be achieved following administration of high doses of dexamethasone (Buonavoglia et al., 1996). CpHV-1 infection is distributed worldwide although it is responsible for major economical losses in Mediterranean countries (Thiry et al., 2006). The CpHV-1 shares many biological similarities with human herpesvirus 2 (HHV-2) and although many reports have shown that mice could be used to study

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HHV-2 infection (reviewed in Ferenczy, 2007) others have shown that the CpHV-1/goat is a useful model to study HHV-2 infection in humans (Tempesta et al., 1999a,b, 2000, 2007c). Thus, implementation of the studies on CpHV-1 will also provide benefit to human medicine where vaccines against HHV-2 are urgently needed (Ferenczy, 2007). Although some data has accumulated on the pathogenesis and latency of CpHV-1 infection, there is limited information on the immune responses to CpHV-1 (Tempesta et al., 2005, 2007b) and no information is available on IgG subclasses.

Cattle have three transcriptionally active genes encoding three IgG subclasses (Rabbani et al., 1997), namely IgG1, IgG2, and IgG3; sheep and goats also seem to have the same genes (Butler, 1998; Micusan and Borduas, 1977) but the lack of commercially available antibodies specific to IgG3 dampens the elucidation of the functional role of the third IgG subclass in these ruminants

Caprine IgG1 and IgG2 responses have been studied by western blot analysis (employing cross-reactive anti-bovine IgG subclass antibodies) following genetic vaccination with a caprine arthritis-encephalitis virus envelope gene (Cheevers et al., 2000) and in *Chlamydophila psittaci* infection where it is important to determine the profile of the IgG subclass in order to distinguish clinical inapparent infections from overt disease (Schmeer et al., 1987). The aim of the present study was to evaluate the distribution of CpHV-1-specific IgG1 and IgG2 in caprine herpesvirus infection by developing CpHV-1-specific ELISAs. In addition, the contribution of the IgG subclasses to the neutralization activity of sera from naturally and experimentally infected goats was also studied.

2. Materials and methods

2.1. Animals

To select the naturally infected goats, 63 goats of mixed breed, age (range 1–4 years) and sex (10 males, 53 females) were screened for serum IgG by using a CpHV-1-specific ELISA. Among the 63 animals screened, there were 20 seronegatives and 43 seropositives; the latter were used for the subsequent evaluation of serum CpHV-1-specific IgG subclasses. For the experimental infection, four additional goats (females, age 3–4 years) were employed. All the animals included in the study were free of caprine arthritis-encephalitis virus. Blood samples were aseptically obtained from the jugular vein and serum was collected by centrifugation at 2000 rpm for 10 min (Beckman microfuge, Fullerton, USA). Serum samples were stored at −20 °C until tested.

2.2. Virus

The Ba.1 strain of CpHV-1 was used to experimentally infect the animals or to prepare the antigen for the ELISA (Buonavoglia et al., 1996). The virus was propagated by infecting Madin Darby Bovine Kidney cells (MDBK; ATCC-LGC Standards, Milan, Italy) grown on Dulbecco-Minimal Essential Medium (D-MEM; Lonza, Walkersville, USA) supplemented with 10% foetal calf serum (FCS; Lonza). The viral titer was $10^{7.00}$ 50% tissue culture infectious doses ($TCDI_{50}$)/50 μl. The viral suspension was tested for bacterial or fungal contamination.

2.3. Experimental infections

For the intravaginal infection, two goats received 4 ml of CpHV-1 Ba.1 strain ($10^{7.00}$ $TCDI_{50}$/50 μl) in the vagina. For the intranasal infection, two goats received 2 ml per nostril of the same viral suspension as described above. Goats were kept under observation for 2 months and examined daily for general and clinical signs, including body temperature, as described previously (Tempesta et al., 2000, 2007a,b,c). At the indicated time points, goats were bled and sera were collected and stored at −20 °C until tested by ELISA and neutralization assays.

2.4. ELISA for serum IgG, IgG1 and IgG2

To measure CpHV-1-specific IgG responses, an assay in use in our laboratory was employed (unpublished). In particular, 96-well polystyrene plates (Nunc, Roskilde, Denmark) were coated with 100 μl/well of CpHV-1 (total protein content, 25 μg/ml) in carbonated buffer (15 mM Na_2CO_3, 35 nM $NaHCO_3$ [pH 9.6]) and incubated overnight at 4 °C on a shaker. After blocking the wells with 200 μl of 2% gelatin/PBS (Sigma, Milan, Italy) for 2 h at 37 °C, individual serum samples diluted 1:100 in 0.05% Tween 20/PBS (PBS/T) were added to duplicated wells and incubated overnight at 4 °C. After washing, HRP-conjugated rabbit anti-goat IgG (Bethyl, Montgomery, USA) diluted 1:1000 in PBS/T were added to the wells and incubated for 2 h at 37 °C. After final washings and addition of ABTS, the colorimetric reaction was measured at 405 nm with an ELISA plate reader (Biorad, Hercules, USA). The O.D. values were recorded and individual readings were reported by subtracting the O.D. values of negative controls (i.e., negative serum samples) to the individual O.D. values. All serum samples were run under identical conditions and, where possible, on the same microtiter plate. The O.D. of negative control sera or background wells (without serum) was 0.030–0.050.

To measure IgG subclasses, a protocol similar to that employed for total IgG was employed with some modifications: (a) serum samples were diluted 1:10 and incubated overnight at 4 °C; (b) HRP-conjugated sheep anti-bovine IgG1 (diluted 1:100 in PBS/T) or HRP-conjugated sheep anti-bovine IgG2 (diluted 1:50 in PBS/T) were employed as detection antibodies (Bethyl); (c) the latter detection antibodies were incubated overnight at 4 °C. The O.D. values (read at 405 nm) were recorded and individual readings were reported by subtracting the O.D. values of negative controls (i.e., negative serum samples) to the individual O.D. values. All serum samples were run under identical conditions and, where possible, on the same microtiter plate. The O.D. of negative control sera or background wells (without serum) was 0.040–0.050 for IgG1 and 0.045–0.055 for IgG2. Preliminary experiments established that sheep anti-bovine IgG1 or IgG2 cross-reacted with goat IgG subclasses. In addition, we performed serum dilution curves with several sera exhibiting a different ratio IgG1:IgG2; over a large serum dilution