Virus Research 144 (2009) 285–289

Contents lists available at ScienceDirect

Virus Research

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

Short communication

Absence of antibody-dependent, complement-mediated lysis of feline infectious peritonitis virus-infected cells

E. Cornelissen, H.L. Dewerchin, E. Van Hamme, H.J. Nauwynck*

Laboratory of Virology, Faculty of Veterinary Medicine, Ghent University, Salisburylaan 133, 9820 Merelbeke, Belgium

ARTICLE INFO

Article history:
Received 23 December 2008
Received in revised form 23 March 2009
Accepted 31 March 2009
Available online 7 April 2009

Keywords:
Feline infectious peritonitis
Antibody
Complement
Cell lysis
ADCML
Immune evasion

ABSTRACT

Cats infected with virulent feline coronavirus which causes feline infectious peritonitis (FIP) usually succumb to disease despite high antibody concentrations. One of the mechanisms that can help resolving infection is antibody-dependent, complement-mediated lysis (ADCML) of infected cells. ADCML consists of virus-specific antibodies that bind to cell surface expressed viral proteins which result in complement activation and cell lysis. The objective of this study was to determine the sensitivity of FIP-virus (FIPV) infected cells towards ADCML and to examine the role of the accessory proteins 3abc and 7ab in this process. ADCML assays, using FIPV strain 79-1146 and its deletion mutant strain Δ3abc/Δ7ab, were performed on: (i) CrFK cells that show surface-expressed viral antigens, (ii) monocytes without surface-expressed viral proteins due to retention and (iii) monocytes with surface-expressed viral proteins since the antibody-mediated internalization of these proteins was blocked. As expected, no ADCML was detected of the monocytes without surface-expressed viral antigens. Surprisingly, no lysis was observed in the CrFK cells and the monocytes that do show surface-expressed viral proteins, while controls showed that the ADCML assay was functional. These experiments proof that FIPV can employ another immune evasion strategy against ADCML (besides preventing surface expression): the inhibition of complement-mediated lysis. This new evasion strategy is not attributed to the group-specific proteins since lysis of cells infected with FIPV Δ3abc/Δ7ab was not detected.

Feline infectious peritonitis (FIP) is a fatal disease, characterized by fibrinous-granulomatous serositis often with protein-rich effusions in body cavities, granulomatous-necrotising phlebitis and periphlebitis and granulomatous inflammatory lesions in several organs (Weiss and Scott, 1981a,b; Kipar et al., 1998, 2005). The causative agent is a virulent form of the feline coronaviruses (FCoVs) belonging to the family Coronaviridae, order Nidovirales. In vivo, monocytes and tissue macrophages are the target cells and play a central role in the development of the lesions (Kipar et al., 2005). These infected cells should be excellent targets for the immune system to fight the infection. However, in most cases the immune response is not protective and the cat succumbs to the infection. The cell-mediated immunity is believed to be important in control and clearance of the FIP-virus (FIPV) infection if there is an efficient first response to the infection. The humoral immune response is believed to be not protective. High concentrations of neutralizing antibodies are present in cats with end-stage FIP and no difference is seen in the antibody concentration and fluctuations between survivors and non-survivors after a FIPV infection (de Groot-Mijnes et al., 2005).

In general, virus-specific antibodies can help to resolve infection by antibody-mediated lysis of infected cells via cytolytic immune cells with Fc receptor (like NK cells, macrophages or neutrophils) or via complement (Sissons and Oldstone, 1980). The complement system is an immunological defense system and plays a role in both the innate and the adaptive immune response against invading pathogens. Complement consists of serum and membrane-bound proteins which, once activated, can trigger a biochemical cascade of reactions contributing to the eradication of pathogens (Blue et al., 2004). Important complement effector functions are opsonization of pathogens, cytolysis and promoting host inflammatory responses (anaphylatoxin and chemotaxin production) (Janeway et al., 2005). In viral infections, the complement system can be activated by free virus particles and virus-infected cells. Complement can inactivate free virus in the presence or absence of antibodies. Opsonization of the virus with complement proteins can promote phagocytosis, virolysis and interference with attachment, internalization or uncoating of the virions (Hirsch, 1982). Cells infected with enveloped viruses can be lysed by complement in the presence of antibodies if newly synthesized viral glycoproteins are expressed at the plasma membrane of the infected cell. Virus-specific antibodies can then bind to these surface-expressed proteins and thereby activate the complement system. Eventually, this results in cell death (Sissons and Oldstone, 1980). This

* Corresponding author. Tel.: +32 9 264 73 73; fax: +32 9 264 74 95.
E-mail address: hans.nauwynck@ugent.be (H.J. Nauwynck).

0168-1702/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.virusres.2009.03.017

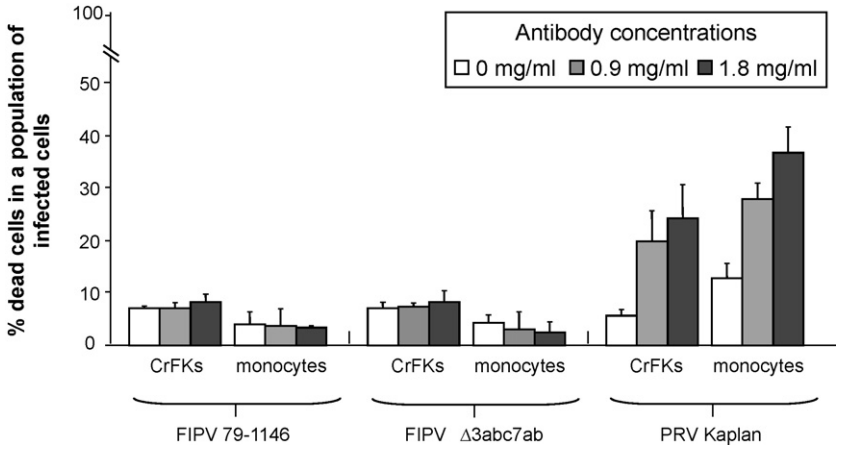

Fig. 1. Antibody-dependent, complement-mediated lysis (ADCML) assay on CrFK cells and monocytes infected with FIPV strain 79-1146, its deletion mutant FIPV Δ3abc/Δ7ab and pseudorabies virus (PRV) strain Kaplan as a control. Different concentrations of virus-specific antibodies were used.

process is called antibody-dependent, complement-mediated lysis (ADCML).

Recently, we described for FIPV two processes that inhibit the expression of viral proteins at the plasma membrane of in vitro infected monocytes. Namely, the retention of viral proteins in infected cells and the antibody-mediated internalization of surface-expressed viral proteins. Both processes result in the clearance of all detectable viral antigens from the plasma membrane of infected cells (Dewerchin et al., 2005; Dewerchin et al., 2006). FIPV-infected monocytes/macrophages isolated from naturally infected cats do not express viral proteins at their plasma membrane either (Cornelissen et al., 2007). Absence of viral proteins in the plasma membrane of infected monocytes can protect the infected cells from efficient ADCML. This has been described for pseudorabies virus (PRV), equine herpesvirus-1 (EHV-1) and porcine reproductive and respiratory syndrome virus (PRRSV) (van der Meulen et al., 2003; Van de Walle et al., 2003; Costers et al., 2006).

The objective of this study was to determine if there is efficient ADCML of FIPV-infected cells that show surface-expressed viral antigens, since this would open treatment possibilities based on inhibiting antibody-mediated internalization of surface-expressed viral antigens. Furthermore, the role of the accessory proteins 3abc and 7ab was assessed in this context.

ADCML assays with Crandell feline kidney cells (CrFKs) and peripheral blood monocytes were performed. CrFKs were seeded in six-well plates (Nunc) and cultivated in MEM-medium containing 5% fetal bovine serum (FBS), 2% lactalbumine, 0.3 mg/ml glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin and 0.1 mg/ml kanamycin. Peripheral blood monocytes were isolated from feline coronavirus, feline leukemia virus and feline immunodeficiency virus negative cats as described previously (Dewerchin et al., 2005). They were cultivated in six-well plates in RPMI 1640-medium containing 10% FBS, 0.3 mg/ml glutamine, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.1 mg/ml kanamycin, 10 U/ml heparin, 1 mM sodium pyruvate and 1% non-essential amino acids 100× (Gibco BRL). Cells were infected with FIPV type II strain 79-1146 or with its attenuated deletion mutant virus strain FIPV Δ3abc/Δ7ab at a multiplicity of infection of 1. Both viruses were kindly provided by Dr Rottier (Faculty of Veterinary Medicine, Utrecht University, The Netherlands). The deletion mutant strain is the FIPV strain 79-1146 from which the open reading frames 3abc and 7ab were deleted, using reverse genetics (Haijema et al., 2004).

The CrFKs and the monocytes were mechanically detached (by gently pipetting up and down) from the wells at 18 h post-inoculation (hpi) and 12 hpi, respectively, to perform the assays in suspension. The cells were incubated for 1 h with FIPV-specific polyclonal antibodies (pAbs) (0, 0.9 and 1.8 mg/ml) or antibodies purified from FIPV-negative serum (1.8 mg/ml). The FIPV-specific pAbs originated from cats infected with FIPV 79-1146 and were provided by Dr Rottier. The FIPV-negative serum was derived from an FCoV-negative cat (IPMA antibody titer <20). Both pAbs had been purified using protein A-Sepharose (Amersham Biosciences). The cells were washed and incubated with 5% complement (non-inactivated serum of a FCoV-negative cat). Then, the cells were stained with ethidium monoazide bromide (EMA) (Molecular Probes) to label dead cells, fixed with 3% paraformaldehyde (VWR), permeabilized with 0.1% saponin (Sigma) and stained with specific monoclonal antibodies (mAbs) against FIPV nucleocapsid (N) protein and membrane (M) protein, followed by FITC-labeled goat anti-mouse IgG (Molecular Probes) to identify FIPV-infected cells. The mAb recognizing the M and N protein were produced and characterized in our laboratory. Nuclei were stained with Hoechst 33342 (Molecular Probes). Dead infected cells were counted using fluorescence microscopy. For the monocytes, the antibody-induced internalization was inhibited by pre-treatment for 30 min with myosin light chain kinase inhibitor and inclusion of the inhibitor during antibody and complement incubation (Dewerchin, 2008).

Since antibodies must be bound to the cell before ADCML can occur, an immunofluorescent staining was performed to determine the presence of the antibodies on the surface of the infected cells. Cells were fixed with 3% paraformaldehyde after incubation with FIPV-specific pAbs. Antibodies were stained with FITC-labeled goat anti-cat IgG (Sigma). After permeabilization with 0.1% saponin, infected cells were stained with N- and M-specific mAbs and Texas Red-labeled goat anti-mouse IgG (Molecular Probes). Nuclei were stained with Hoechst 33342 (Molecular Probes).

Two control assays were performed to verify the functionality of the ADCML assay: (i) a FIPV neutralization assay to detect a higher neutralization in the presence of complement in order to confirm the activity of the feline complement in combination with the FIPV-specific pAbs and (ii) an ADCML assay on pseudorabies virus (PRV)-infected feline monocytes and CrFKs to confirm the activity of the feline complement, the sensitivity of the cells to ADCML and to exclude possible interference of the used media. The ADCML assay was performed as described above using PRV strain Kaplan, feline pAbs against PRV (derived from a Geskypur (Merial) vaccinated FCoV-negative cat, according to manufacturer's instructions), feline complement and FITC-labeled PRV-specific pAbs to identify infected cells. It has been described that PRV-infected porcine macrophages with surface-expressed viral antigens are sensitive to ADCML (Van de Walle et al., 2003).