INSTITUT PASTEUR

Microbes and Infection 9 (2007) 1530–1537

www.elsevier.com/locate/micinf

Original article

Natural antibody–complement dependent neutralization of bovine herpesvirus 4 by human serum

Bénédicte Machiels [a], Laurent Gillet [a], Sieberth Do Nascimento Brito [a], Pierre Drion [b], Cédric Delforge [a], Yannick Nizet [c], Pierre Gianello [c], Christophe Bona [a], Bérénice Costes [a], Nicolas Markine-Goriaynoff [a], Alain Vanderplasschen [a,*]

[a] *Immunology–Vaccinology (B43b), Department of Infectious and Parasitic Diseases, Faculty of Veterinary Medicine, University of Liège, Bvd de Colonster 20, B-4000 Liège, Belgium*

[b] *Animal facility (B23), University of Liège, B-4000 Liège, Belgium*

[c] *Experimental Immunology Unit, Université de Louvain, Faculté de Médecine, Department of Pathology, Cliniques Universitaires Saint-Luc, Brussels, Belgium*

Received 17 May 2007; accepted 23 August 2007
Available online 31 August 2007

Abstract

In contrast to most gammaherpesviruses, *Bovine herpesvirus 4* (BoHV-4) has a broad range of host species both *in vitro* and *in vivo*. Several *in vitro* studies demonstrated that some human cell lines are sensitive or even permissive to BoHV-4. These observations led to the hypothesis that cross-species transmission of BoHV-4 could lead to human infections. In the present study, we investigate the sensitivity of BoHV-4 to neutralization by naïve human sera in order to determine if humans exhibit innate anti-viral activities against this virus. Our results demonstrate that human sera from naïve individuals, in contrast to the sera of naïve subjects from various animal species, neutralize BoHV-4 efficiently. A series of complementary experiments were performed to unravel the mechanism(s) of this neutralization. The data obtained in this study demonstrates that human serum neutralizes BoHV-4 in a complement dependent manner activated by natural antibodies raised against the Galα1-3Galβ1-4GlcNAc-R epitope expressed by bovine cells.

Keywords: Bovine herpesvirus 4; Innate anti-viral immunity; Virus neutralization; Complement; Natural antibodies

1. Introduction

Bovine herpesvirus 4 (BoHV-4) belongs to the *Herpesviridae* family, *gammaherpesvirinae* subfamily, *rhadinovirus* genus [1]. BoHV-4 has been isolated throughout the world from both healthy cattle as well as from those exhibiting a variety of diseases. In contrast to most gammaherpesviruses, BoHV-4 can replicate in a broad range of host species both *in vitro* and *in vivo*. Several *in vitro* studies demonstrated that some human cell lines are sensitive or even permissive to BoHV-4 [2–4]. The sensitivity of human cells to BoHV-4 infection has been investigated to address two main questions.

The observation that human cells can support BoHV-4 infection led to the hypothesis that field strains of BoHV-4 could represent a danger for human health [2,4]. BoHV-4 could be potentially harmful to humans either by replicating in permissive cells and/or by protecting non-permissive persistently infected cells from apoptosis [4]. *In vivo*, the latter phenomenon could allow the infected cells to accumulate mutations leading eventually to transformation [4]. Importantly, this

Abbreviations: BoHV-4, *Bovine herpesvirus 4*; EGFP, enhanced green fluorescent protein; Galα1-3Gal, Galα1-3Galβ1-4GlcNAc-R; GT, α1-3-galactosyltransferase; MDBK, Madin Darby Bovine Kidney; MEM, minimal essential medium; MBL, mannan-binding lectin; Mab, mouse monoclonal antibody.

* Corresponding author. Tel.: +32 4 366 42 64; fax: +32 4 366 39 08.
E-mail address: a.vdplasschen@ulg.ac.be (A. Vanderplasschen).

1286-4579/$ - see front matter © 2007 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.micinf.2007.08.007

property has been shown to play an important role in the oncogenesis induced by several gammaherpesviruses [5,6]. The risk of a viral cross-species transmission relies on several factors such as, the prevalence of the virus in the environment, the existence of events permitting the transmission of the virus, and finally, the capacity of the virus to infect and to persist in the non-natural host. In relation to these factors, several observations support the existence of a risk of BoHV-4 transmission to humans. Firstly, BoHV-4 is highly prevalent in the cattle population and no eradication scheme is directed against this virus. Secondly, there are many factors that increase the exposure of this virus from cattle to humans: (i) BoHV-4 is frequently isolated from bovine serum [7] which is abundantly used in food and pharmacological preparations [8], making human contamination by BoHV-4 possible by enteral or parenteral routes; (ii) BoHV-4 has been found in the milk of cows with mastitis as well as from apparently healthy cows, suggesting possible human contamination by oral route through milk ingestion [9,10]. The latter risk is drastically reduced by the sensitivity of BoHV-4 to milk pasteurization [11]; and (iii) infected animals excrete BoHV-4 in nasal and vaginal discharges both after primary infection and after reactivation [12] making human contamination possible resulting from direct contact with infected cattle. However, to date there are no reported cases of human infection with BoHV-4.

The ability of BoHV-4 to infect human cells has also been investigated to address the potential of BoHV-4 recombinant strains for viro-oncolytic treatment of human cancer. Recently, we reported the property of BoHV-4 to induce, *in vitro* and *in vivo*, apoptosis of some human carcinomas [13]. *In vivo* assays were performed in nude mice, and consequently, in absence of human innate immunity.

Human innate immunity against BoHV-4 could be crucial for both questions presented above resulting in beneficial or undesirable effects. Indeed, on the one hand, natural immunity could prevent human infection after contamination by BoHV-4 field strains. On the other hand, the potential of modified BoHV-4 strains as viro-oncolytic treatment of cancer could be drastically reduced by human innate anti-viral immunity.

Innate humoral immunity against viruses can be mediated by complement. Activation of complement on virions can occur independently of antibodies [14] or be the consequence of natural antibody binding on virions (Antibody–complement dependent viral neutralization) [15,16]. The serum of humans, apes and Old World monkeys contain high levels of natural antibodies specific for the carbohydrate moiety Galα1-3Galβ1-4GlcNAc-R (Galα1-3Gal) [16]. This moiety is a product of the α-1-3-galactosyltransferase (GT) enzyme that adds a terminal galactose onto glycoproteins and glycolipids in a specific α1-3 linkage. Humans, apes and Old World monkeys do not express the Galα1-3Gal moiety as the consequence of an ancestral inactivation of their *GT* gene. The non-expression of this moiety resulted in loss of immune tolerance and production of anti-Galα1-3Gal antibodies as a presumed consequence of bacterial exposure [16]. In contrast, New World monkeys, prosimians and non-primate mammals express a functional GT enzyme which produces the Galα1-3Gal epitope as a self antigen, preventing these species from producing anti-Galα1-3Gal antibodies. Human serum contains high level of IgG and IgM against Galα1-3Gal [17]. Anti-Galα1-3Gal constitutes approximately 1% of circulating IgG. It has been shown for few enveloped viruses that their passage through cells expressing Galα1-3Gal make them potential targets for natural human antibodies against Galα1-3Gal and eventually sensitive to antibody dependent complement neutralization [18–22].

In the present study, we investigate the sensitivity of BoHV-4 to neutralization by naïve human serum in order to determine if humans exhibit innate anti-viral activities against this virus. Our results demonstrate that human sera from naïve individuals, in contrast to the sera of naïve subjects from various animal species, neutralize BoHV-4 efficiently. A series of complementary experiments demonstrated that human serum neutralizes BoHV-4 through activation of complement by natural antibodies raised against bovine host cell derived epitopes. The Galα1-3Gal epitope expressed by bovine cells was proved to play a key role in this neutralization. This study is the first to support the role of human natural anti-Galα1-3Gal antibodies as an innate immune mechanism against a gammaherpesvirus. The present study also raises questions that must be considered when developing BoHV-4 recombinants as viro-oncolytic vectors.

2. Materials and methods

2.1. Cells and virus

Madin Darby Bovine Kidney (MDBK) (ATCC CCL 22) and VERO (ATCC CCL 81) (African green monkey) cells were cultured following the recommendations of the ATCC (http://www.atcc.org). The BoHV-4 V. test EGFP *Xho* I recombinant strain was used throughout this study. This strain carries an enhanced green fluorescent protein (EGFP) expression cassette under control of the human cytomegalovirus *IE* gene promoter/enhancer in a region of the viral genome which does not contain any open reading frames. Comparison of the parental V. test strain and the derived V. test EGFP *Xho* I revealed no difference in viral replication both by single round and multiple step assays [4]. Virus grown on MDBK or VERO cells was semi-purified and stored as described previously [13].

2.2. Complement reagents

Serum was collected as a source of complement from human volunteers with different ABO blood groups and from several animal species (Table 1). The sera were treated as described previously to preserve complement activity, aliquoted and stored at −80 °C [23]. Complement activity of stored sera was controlled using the AH50 assay as described elsewhere [24]. This assay relies on the lysis of unsensitized rabbit erythrocytes by sample serum in a buffer containing the calcium chelator EGTA, thus preventing the activation of the calcium-dependent classical pathway. The results are expressed