Veterinary Immunology and Immunopathology 150 (2012) 1–9

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

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

Research paper

Induction of a systemic antiviral state *in vivo* in the domestic cat with a class A CpG oligonucleotide

Céline Robert-Tissot*, Marina L. Meli, Barbara Riond, Regina Hofmann-Lehmann, Hans Lutz

Clinical Laboratory, Vetsuisse Faculty, University of Zurich, Winterthurerstr. 260, CH-8057 Zurich, Switzerland

ARTICLE INFO

Article history:
Received 13 December 2011
Received in revised form 21 July 2012
Accepted 30 July 2012

Keywords:
Domestic cat
CpG oligonucleotide
Antiviral state
Prophylaxis
Viral infections

ABSTRACT

The evolution of cats as a solitary species has pressured feline viruses to develop highly efficient transmission strategies, the ability to persist within the host for long periods of time and the aptitude to adapt to natural and vaccine-induced immunological pressures. These characteristics render feline viruses particularly dangerous in catteries, shelters and rescue homes, were cats from different backgrounds live in close proximity. The possibility to induce short-term resistance of newcomer cats to a broad variety of viruses could help prevent the dissemination of viruses both within and outside such facilities. Oligonucleotides (ODN) containing unmethylated cytosine phosphate guanosine (CpG) motifs stimulate innate immune responses in mammals. We have previously shown that ODN 2216, a class A CpG ODN, promotes the expression by feline immune cells of potent antiviral molecules that increase resistance of feline fibroblastic and epithelial cell lines to five common feline viruses. With the aim to test the safety and extent of the biological effects of ODN 2216 in the domestic cat, we performed an initial *in vivo* experiment in which two cats were injected the molecule once subcutaneously and two additional cats received control treatments. No side effects to administration of ODN 2216 were observed. Moreover, this molecule induced the expression of the myxovirus resistance (Mx) gene, a marker for the instigation of innate antiviral processes, in blood as well as in oral, conjunctival and rectal mucosa cells, indicating systemic biological activity of the molecule with protective potential at viral entry sites. Mx mRNA levels were already elevated in blood 6 h post injection of ODN 2216, reached peak levels within 24 h and returned to basal values by 96–192 h after administration of the molecule. Similar induction patterns were observed in all analyzed mucosal cells. Plasma collected from treated cats at regular intervals until 96–192 h could moreover induce Mx mRNA expression in fcwf-4 cells and increase resistance of these cells to feline calicivirus inoculation. Finally, Mx mRNA levels measured in blood correlated with the degree of viral inhibition that was induced by plasma from the same cat and the same experimental time point. Our results altogether underline the promising potential of ODN 2216 in promoting antiviral defense mechanisms and inducing temporary resistance to viral infections *in vivo* in the domestic cat.

1. Introduction

Feline viruses have evolutionarily acquired elaborate strategies to persist within their host population. Although the domestic cat is a social species, its ancestor the African wildcat (*Felis silvestris lybica*) led a solitary way of life

* Corresponding author. Tel.: +41 44 635 8378; fax: +41 44 635 8923.
E-mail address: crobert@vetclinics.uzh.ch (C. Robert-Tissot).

0165-2427/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetimm.2012.07.015

(Driscoll et al., 2009). As a result, feline viruses possess very efficient transmission strategies enabling them to infect susceptible individuals upon the rare contact between animals (Pontier et al., 2009). Additionally, most of these pathogens apply the "hit and stay" strategy, in that they induce asymptomatic, latent and/or persistent infections, and remain for a long time within the host after infection (Hilleman, 2004). Feline viruses have conserved their opportunistic behavior over the course of time, and modern animal welfare gives a free way to these pathogens, which readily spread to every individual living in a group.

The biological properties of feline viruses are today particularly problematic in catteries and shelters, where the infectious pressure is significantly increased by high social contact rates among stressed individuals with different medical backgrounds (Helps et al., 2005; Pedersen et al., 2004; Prado et al., 2009). Healthy carrier animals threaten to infect susceptible residents, which then further participate in viral dissemination during acute infection. These cats leave the facility with an infection that may be discovered only months or years later, and the connection to a stay at a cattery or shelter is often overseen. Sadly, most feline viruses are fatal and disease management is generally long and tedious (Addie et al., 2009; Lutz et al., 2009; Radford et al., 2009; Thiry et al., 2009; Truyen et al., 2009). Specific therapies are moreover unavailable for most feline viral diseases, and when existent, they tend to be very costly. An emphasis should therefore be set on effective prophylaxis. Efficient protection by vaccination, however, requires lengthy protocols and quarantine of naive animals, which remain logistically difficult in overcrowded shelters. Furthermore, as selective pressure has altered the field strain variety of feline viruses, the efficacy of available vaccines has been questioned (Hurley et al., 2004; Radford et al., 2006). The possibility to transiently increase resistance to a broad spectrum of viruses in cats placed in a cattery or shelter could restrict viral spread within the facility and significantly contribute to the well being of cats and their owners. We like to refer to this idea as the "teflonization" of the domestic cat, in analogy to Ronald Reagan, the "teflon president" (Thomas et al., 1984). Just as strong critics during his administration seemed to have no effect on Reagan's popularity, feline viruses could not affect the teflonized cat.

Oligonucleotides containing unmethylated cytosine-guanosine pairs (CpG ODN) are known to stimulate the innate branch of the immune system of mice, primates and many domestic species (Abel et al., 2005; Kamstrup et al., 2001; Kurata et al., 2004; Mena et al., 2003; Rankin et al., 2001) and have made their way to human clinical trials (Gupta and Agrawal, 2010; Klinman et al., 2009; Murad and Clay, 2009; Vollmer and Krieg, 2009). According to sequence, backbone structure and immunological effect, several classes of CpG ODN have been defined, among which classes A and B have been most widely studied (Vollmer et al., 2004). While class A CpG ODN (CpG-A) comprise several CpG motifs on an endonuclease-sensitive phosphodiester backbone flanked by synthetic phosphorothioate poly(G) stretches, the CpG motifs in CpG-B molecules are fully embedded in the endonuclease-resistant phosphorothioate backbone. These structural differences are known to affect not only the stability of the CpG ODN classes *in vivo*, but also their cellular targets as well as the ensuing immune responses. Although both classes trigger the Toll-like receptor (TLR) 9, CpG-A more efficiently induce production of type I interferon (IFN) by plasmacytoid dendritic cells (pDC), while CpG-B are known to stimulate monocytes and B cells.

To date only few studies report effects of CpG ODN in cats; all indicate these molecules possess the potential to mount advantageous immune responses in the context of viral infections. In this way, feline immune cells displayed increased proliferation after stimulation with both class A (Robert-Tissot et al., 2012) and class B (Wernette et al., 2002) CpG ODN. Furthermore, addition of a class B CpG ODN as allergen adjuvant in a feline asthma model promoted the induction of a T helper (Th) 1 response, which involves immune cells with the capability to interfere with viral propagation (Reinero et al., 2008). Several class A CpG ODNs were also shown to promote the expression of IFNγ, a typical Th1 cytokine (Satoh et al., 2011). In an extensive study, we recently reported that ODN 2216, the prototype CpG-A, could induce robust antiviral immune responses in the domestic cat *in vitro* (Robert-Tissot et al., 2012). Feline peripheral blood mononuclear cells (PBMCs) stimulated with this molecule displayed stronger proliferation capacities, higher presence of surface co-stimulatory molecules and enhanced expression of potent antiviral cytokines, including various subtypes of feline IFNα and IFNω, which belong to the type I IFN family. Soluble molecules in PBMC supernatants further enhanced resistance of target feline cell lines to inoculation with five common feline viruses including the feline calicivirus (FCV), herpesvirus (FHV), coronavirus (FCoV), parvovirus (FPV) and leukaemia virus (FeLV). Moreover, we found that higher expression level of Myxovirus resistance (Mx) GTPase, a gene directly and solely induced by type I IFN (Haller and Kochs, 2011), strongly correlated with decreased susceptibility of target cells for replication of these viruses.

In order to further investigate the adequacy of ODN 2216 as a candidate for prophylactic induction of broad-spectrum antiviral protection in the domestic cat, we conducted an initial *in vivo* experiment. In the present study, we assess the safety of ODN 2216 for clinical usage, and measure the extent and kinetics of its biological effects after a single injection. Finally, we evaluate the *in vivo* antiviral potential of this molecule.

2. Material and methods

2.1. Cats

Four male castrated specific pathogen free (SPF) cats purchased from Liberty Research Inc. (Waverly, NY, USA) were included in this study. The SPF status of the animals was verified as previously described (Museux et al., 2009). At the time of the experiment, the cats were 40 months old. They were kept in an animal-friendly environment under optimal ethological conditions at our facility at the Vetsuisse Faculty, University of Zurich (Geret et al., 2011). The present study was officially approved by the