Veterinary Microbiology 150 (2011) 239–247

Contents lists available at ScienceDirect

Veterinary Microbiology

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

Phylogenetic characterisation of naturally occurring feline immunodeficiency virus in the United Kingdom

A. Samman [a], E.L. McMonagle [a], N. Logan [a], B.J. Willett [a], R. Biek [a,b], M.J. Hosie [a,*]

[a] MRC - University of Glasgow Centre for Virus Research, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bearsden, Glasgow G61 1QH, United Kingdom
[b] Boyd Orr Centre for Population and Ecosystem Health, College of Medical, Veterinary and Life Sciences, University of Glasgow, Bearsden, Glasgow G61 1QH, United Kingdom

ARTICLE INFO

Article history:
Received 7 December 2010
Received in revised form 20 January 2011
Accepted 24 January 2011

Keywords:
Feline immunodeficiency virus (FIV)
Vaccine
Phylogeny

ABSTRACT

Feline immunodeficiency virus (FIV) is a significant pathogen of domestic and non-domestic felids worldwide. In domestic cats, FIV is classified into five distinct subtypes (A–E) with subtypes A and B distributed most widely. However, little is known about the degree of intrasubtype viral diversity and this may prove critical in determining whether monovalent vaccines are likely to protect against FIV strains within a single subtype. Here, we characterise novel *env* sequences from 47 FIV strains recovered from infected cats in the United Kingdom and its environs. Phylogenetic analyses revealed that all bar one sequence belonged to subtype A, the predominant subtype in Western Europe. A single sequence was identified as a likely subtype A/C recombinant, intriguing given that subtype C does not appear to exist in either the UK or North Western Europe and suggestive of a recombination event predating its introduction into the UK. Subtype A strains from the UK were not significantly differentiated from representative subtype A isolates found elsewhere suggesting multiple introductions of FIV into the country. Divergence among isolates was comparable to that observed for subtype A isolates worldwide, indicating that FIV in the UK covers the full spectrum of subtype A diversity seen globally. This study demonstrates that while subtype A is predominant in the UK, novel introductions may result in the emergence of novel subtypes or intersubtype recombinants, potentially circumventing vaccine strategies. However, the dominance of subtype A suggests that the development of a regional or subtype-specific protective vaccine for the UK could be achievable.

1. Introduction

Feline immunodeficiency virus (FIV) is a widespread pathogen of the domestic cat and infection results in a progressive immune dysfunction similar to AIDS in human immunodeficiency virus (HIV) infection. Since its discovery in 1986 from a cat with an immunodeficiency like syndrome (Pedersen et al., 1987), FIV has been recognized widely as the feline equivalent of human immunodeficiency virus (HIV) and both viruses share significant physical, biochemical and pathogenic features (Johnson et al., 1994). Such are the similarities between FIV and HIV that FIV serves as a valuable animal model for both prophylactic and therapeutic studies of HIV (Elder and Phillips, 1995) as well as being the only non-primate lentivirus that induces an AIDS-like syndrome in its natural host (reviewed in Willett et al., 1997).

Like other retroviruses, FIV has high mutation rate, mainly due to the error-prone reverse transcriptase (Shankarappa et al., 1999); accordingly, diverse viral variants emerge continually in the infected host. Since the *env* gene is the key determinant of viral diversity among FIV (Olmsted et al., 1989), FIV phylogeny studies have focussed mainly on *env* sequences. According to the nucleotide

* Corresponding author. Tel.: +44 141 330 2193.
E-mail address: margaret.hosie@glasgow.ac.uk (M.J. Hosie).

0378-1135
doi:10.1016/j.vetmic.2011.01.027

sequence diversity of the V3–V5 region of *env*, FIV is classified currently into five distinct phylogenetic subtypes, designated A to E (Sodora et al., 1994; Pecoraro et al., 1996); subtypes A and B are the most commonly occurring worldwide (Martins et al., 2008). Furthermore, similar to HIV-1, several inter-subtype FIV recombinants have been recognized in natural populations following co-infection; inter-subtype recombinants A/B, A/C and B/D have been identified (Bachmann et al., 1997; Hayward and Rodrigo, 2008).

Phylogenetic studies on FIV sequences have revealed significant heterogeneity (up to 30%) in the sequence of the *env* gene of FIV isolates worldwide (Burkhard and Dean, 2003) similar to that estimated for HIV-1 (Rong et al., 2007). Subtype A isolates are common in Australia, New Zealand, western United States, South Africa and north-western Europe (Bachmann et al., 1997; Steinrigl and Klein, 2003; Kann et al., 2006a,b, 2007a,b). Subtype B isolates have been identified in the central and eastern United States, central Europe, Brazil and eastern Japan (Sodora et al., 1994; Kakinuma et al., 1995; Pistello et al., 1997; Nishimura et al., 1998; Steinrigl and Klein, 2003; Martins et al., 2008). Subtype C has been recognized in Canada, New Zealand and southeast Asia (Uema et al., 1999; Nakamura et al., 2003; Reggeti and Bienzle, 2004; Hayward et al., 2007). Finally, subtypes D and E are infrequent but were identified originally in southwestern Canada and Japan, and Argentina, respectively (Pecoraro et al., 1996; Nishimura et al., 1998).

Such heterogeneity in *env* gene sequence poses problems for the design of a broadly protective vaccine. It has been reported previously that effective protection was obtained following a homologous FIV challenge using an inactivated whole virus vaccine, although, despite the relative success of Fel-O-Vax against heterologous subtype B isolates (Pu et al., 2005), protection did not extend to a heterologous challenge (Dunham et al., 2006). Such different outcomes highlight the impact of genetic diversity on vaccine strategies against FIV and the importance of assessing the genetic diversity of local subtypes for vaccine development, or before introducing a commercial vaccine to a particular geographical area. Furthermore, identification of the predominant strains in a given region is necessary in order to develop appropriate reagents for the molecular diagnosis of FIV infection (Leutenegger et al., 1999; Hosie et al., 2002).

In order to understand better the degree of intrasubtype viral diversity and the likelihood of a monovalent subtype A FIV vaccine offering broad intrasubtype immunity, we investigated the diversity of FIV within the United Kingdom, a country in which FIV infection is prevalent and where evidence to date suggests that a single viral subtype may dominate.

2. Materials and methods

2.1. Blood samples, PBMC collection and DNA extraction

Blood samples were collected from 47 FIV sero-positive samples from naturally infected cats submitted to the Companion Animals Diagnostic Services at the University of Glasgow. Cats were either companion pets or had been placed into re-homing shelters. Blood samples were centrifuged at 2000 rpm for 10 min and the plasma removed. Cell pellets were diluted in 1 ml phosphate buffered saline (PBS) and transferred to 20 ml pre-warmed red blood cell lysis buffer (0.88% NH_4Cl, 10 mM; pH 7.4) and incubated at room temperature for 5 min (red blood cell lysis). The intact white blood cells were then pelleted by centrifugation at 1000 rpm for 5 min. The supernatant was then discarded and the pelleted cells washed twice by centrifugation through ice-cold PBSA buffer (1% bovine serum albumin in PBS). 10^7 cells were used for DNA extraction using QIAamp DNA Blood Mini Kit (Qiagen, Crawley, UK), as per the manufacturer's protocol.

2.2. Cloning of FIV env genes and nucleic acid sequence determination

Env genes were amplified by using the polymerase chain reaction (PCR) using a high fidelity enzyme mix (Expand High Fidelity PCR system, Roche Diagnostics Ltd., Burgess Hill, UK) and the degenerate primers 1F4 5′-TGTAATCAACG(CT)TTTGT(AG)TC-3′ and 1R4 5′-CCAA-TA(AC)TCCCAGTCCACCCTT-3′, primers we have found previously to amplify laboratory strains of viral subtypes A, B and C. The FIV env genes were then cloned into the VR1012 eukaryotic expression vector (Vical Inc., San Diego, CA, USA) and their nucleic acid sequences determined using a BigDye Terminator v1.1 kit (Applied Biosystems, Warrington, UK). Sequencing was performed by using Applied Biosystems 3730xl genetic analyser. Raw chromatographic data were analysed by using 'Contig Express' sequence analysis software within the Vector NTI suite of programs (Invitrogen Ltd., Paisley, UK).

2.3. Multiple sequence alignments and phylogenetic analyses

Nucleotide sequence analysis was performed on a 651 nucleotide fragment spanning the V3–V5 region of *env* gene. The generated consensus included sequences of the 47 isolates included in the study, as well as selected representative sequences from subtypes A–E. Multiple alignment was performed using Clustal X (version 2.0) (Larkin et al., 2007), followed by manual adjustment. Alignments were translated and the resulting amino acid-based alignments used as an exact guide for re-positioning of improper gapping, particularly, where sequences were different in length. Final alignments are available from the authors upon request. DNA distance matrices were calculated with Paup (Swofford, 1993; Wilgenbusch and Swofford, 2003) under a GTR+I+G model, selected in Modeltest (Posada and Crandall, 1998) and parameterized using Maximum Likelihood (ML). A Bayesian phylogeny was estimated in MrBayes under a SDR06 model of evolution (Huelsenbeck and Ronquist, 2001) based on two independent runs of 10 Million generations, with samples taken every 2000 generations. Because initial analyses indicated a problem with inflated branch lengths (Brown et al., 2010), a ML tree (generated in Paup) was added as a starting tree and the branch length prior was adjusted according to the formula provided in (Brown et al., 2010).