Virology 462-463 (2014) 309–317

Contents lists available at ScienceDirect

Virology

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

Lineage diversification of pigeon paramyxovirus effect re-emergence potential in chickens

Yee Ling Chong [a,b,*], Oekyung Kim [a], Mary Poss [a,*]

[a] Center for Infectious Disease Dynamics, Department of Biology, The Pennsylvania State University, University Park, PA 16802, USA
[b] Department of Zoology, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

ARTICLE INFO

Article history:
Received 11 February 2014
Returned to author for revisions
8 June 2014
Accepted 9 June 2014
Available online 8 July 2014

Keywords:
Paramyxovirus
Genetic-phenotypic association
Multivariate analysis

ABSTRACT

Genotype VI-paramyxovirus (GVI-PMV1) is a major cause of epidemic Newcastle-like disease in Columbiformes. This genotype of avian paramyxovirus type 1 has diversified rapidly since its introduction into the US in 1982 resulting in two extant lineages, which have different population growth properties. Although some GVI-PMV1s replicate poorly in chickens, it is possible that variants with different replicative or pathogenic potential in chickens exist among the genetically-diverse GVI-PMV1s strains. To determine if variants of Columbiform GVI-PMV1 with different phylogenetic affiliations have distinct phenotypic properties in chickens, we investigated the replicative properties of 10 naturally circulating pigeon-derived isolates representing four subgroups of GVI-PMV1 in primary chicken lung epithelial cells and in chicken embryos. Our data demonstrate that GVI-PMV1 variants have different infection phenotypes in their chicken source host and that properties reflect subgroup affiliation. These subgroup replicative properties are consistent with observed dynamics of viral population growth.

Introduction

Genotype-VI paramyxovirus (GVI-PMV1) arose from cross-species transmissions from chickens to pigeons and now has strong host fidelity to pigeons (Chong et al., 2013). GVI-PMV1 strains cause systemic infection in pigeons resulting in respiratory disease (Toro et al., 2005) and with notable symptoms in the central nervous system and gastrointestinal tract (Barber et al., 2010). GVI-PMV1 is most frequently detected in Columbiformes (*i.e.* pigeons and doves) populations (Kim et al., 2008) but continues to cause sporadic outbreaks in chickens (Abolnik et al., 2008; Hassan et al., 2010; Pedersen et al., 2004). Despite possessing the genetic signature for virulence at the F gene cleavage site, GVI-PMV1 isolates typically replicate poorly and are not virulent in chickens in experimental studies (Meulemans et al., 2002). The virulence potential of GVI-PMV1 in chickens is determined by virus strain as well as by the age of chickens, route and intensity of viral inoculations used in the experimental settings (Gelb et al., 1987; King, 1996; Pearson et al., 1987; Toro et al., 2005). For example, the consequences of infection by different GVI-PMV1 strains range from mild respiratory disease to neurotropic disease and mortality in experimentally infected chickens (Gelb et al., 1987; Pearson et al., 1987; Toro et al., 2005). Serial passage in chickens or embryonated eggs increased virulence (Dortmans et al., 2011; Fuller et al., 2007; Kommers et al., 2001) and enhanced the replication of GVI-PMV1 (Dortmans et al., 2011), and in one study isolates recovered from experimentally passaged virus in chickens had only three mutations in two viral genes, P and L (Dortmans et al., 2011). This data suggest that small changes in the viral genome are sufficient to allow GVI-PMV1 infections in chickens.

After the emergence of GVI-PMV1 in European Columbiformes in the 1980s, there were at least three virus introductions to North America. GVI-PMV1 in North American Columbiformes has evolved at a high substitution rate and there is measurable genetic diversity in contemporary strains (Chong et al., 2013). Two extant lineages, GVIbii-*d* and -*g*, have diversified locally; the former has maintained a fairly constant viral population size over time, whereas the later has experienced exponential population growth (Chong et al., 2013). Despite the apparent fidelity of GVI-PMV1 for pigeons, outbreaks in poultry continue to be reported in many countries (Abolnik et al., 2008; Hassan et al., 2010; Mase et al., 2002; Pedersen et al., 2004; Ujvari et al., 2003) and GVI-PMV1 can be isolated from chickens in the absence of an epizootic. It is therefore of substantial interest for both risk assessment and diagnostics to determine if the genetic diversification of the GVIbii-*d* and -*g* lineages derived from the pigeons has

* Corresponding authors.
E-mail addresses: yeelingchong@gmail.com (Y.L. Chong), mposs@bx.psu.edu (M. Poss).

http://dx.doi.org/10.1016/j.virol.2014.06.007
0042-6822/

conferred unique infection phenotypes in chickens, and whether phenotypic properties in chickens are associated with the observed viral population growth patterns that characterize each lineage.

To this end, we assessed the organismal and cellular replication of 10 GVI-PMV1 isolates representing GVIbii-*d* and -*g* lineages obtained from naturally infected pigeons. The complete genomes of these selected isolates were sequenced. Linear discriminant analyses (LDA) were used to determine if infection phenotype correlated with viral genotype. Our data indicate that rapid diversification of a virus in a new host has consequences to infection properties in the original host. Data linking genotype and phenotype of viruses adapting to new hosts is of substantial importance as many important virus infections in humans and animals arise from cross-species transmission.

Results

Evolutionary history of GVI-PMV1 isolates used in this study

Ten GVI-PMV1 isolates obtained from sick pigeons submitted to the Animal Diagnostic Lab were selected based on the phylogenetic affiliations of the F gene (Fig. 1A), and were assigned to subgroups A – D based on bootstrap support. Subgroup A – C are GVIbii-*g* viruses, which are undergoing population expansion, while subgroup D is a member of lineage GVIbii-*d*, which demonstrates constant population size (Fig. 1A, upper inset).

We estimated recombination and selection profiles on these 10 isolates in relation to 51 isolates that were previously described (Chong et al., 2013) (Fig. S1). The phylogenetic affiliations of all isolates were consistent based F (Fig. 1), P, HN and on concatenated genes (Fig. S1), indicating that there was no recombination in this region of the genome (Chong et al., 2010). Using codon-based models and maximum likelihood methods implemented in PAML (Yang, 1997) and the Datamonkey webserver (Pond and Frost, 2005), we demonstrated that the overall d_N/d_S of each of the three genes was less than 1 indicating that purifying selection was predominant. Four codons in the P protein and one codon in F protein were under positive selection (Fig. 1B). Two of the four positively selected codons (*i.e.* 77 and 92) in P were predicted phosphorylation sites and fall in the region of P that overlaps with the small V protein, which is produced by post-transcriptional editing (Steward et al., 1993) (Table S3). In contrast, none of the codons in HN were under positive selection.

Survival kinetics of 10-day old chicken embryos infected with ten GVI-PMV1 isolates

We assessed the relative pathogenic potential of each isolate in ten-day old chicken embryos inoculated *via* the allantoic fluid route by recording the number of embryos that died every 12 h for seven days. The survival rates of chicken embryos infected with each virus differed ($P < 0.001$; Cox proportional hazards regression model), and were partially correlated with their subgroup classification (Fig. 2A). All embryos inoculated with subgroup A, B, and D viruses succumbed to infection within the experiment periods. PA-0725 (the only B subgroup virus) killed all embryos by 114 hpi, which was similar to that observed for the vaccine strain LaSota. The time to death of embryos infected with A and D subgroups ranged from 122–144 hpi. In contrast, embryos inoculated with subgroup C viruses had the longest survival times and there was no embryo mortality associated with na-106 inoculation.

Systemic infection in 18-day old chicken embryos

Chicken embryos have multiple tissues to support virus infection and each is capable of mounting a distinct host innate defense. Embryos are therefore reasonable surrogates for *in vivo* infection. As the 10 isolates of pigeon GVI-PMV1 showed different effects in chicken embryos at early [10–12 days] developmental stages, we further evaluated each virus for the ability to replicate in lung, liver, and spleen of chicken embryos at later developmental stages [18–20 days]. After 48 hpi, all embryos were alive and there were no gross pathological alterations of internal organs.

Viral transcripts from all 10 GVIb viruses and LaSota were detected in both lung and liver tissues. Moreover, all viruses except for PA-0725 and PA-0704 showed higher viral transcripts productions in lung, which is the primary site of infection following amniotic inoculation, compared to liver ($P < 0.05$). Although there were significant differences among the viruses in viral transcript production in lung (i.e production of both MD-0719 and PA-0805 transcripts in lung was significantly higher than na-0106 and PA-0810 ($P < 0.05$)) there was no apparent correlation between transcript production and virus subgroup. However, there were significant effects of both tissue type and virus subgroup on the total viral transcript production ($P < 0.05$; factorial ANOVA test) (Fig. 2B). In liver, PA-0725 (B subgroup) produced a higher level of viral transcripts than na-0101 and PA-0712 (D subgroup), na-0106 and NJ-0717 (C subgroup), and NJ-0607 (A subgroup) [$P \leq 0.01$]. Further, detection of viral transcripts in spleen is indicative of systemic infection. Only PA-0725 consistently dispersed to spleen; PA-0704 and NY-0717 transcripts were each found in spleen in one of seven replicates. While other GVI-PMV1 viruses and the low pathogenic LaSota vaccine strain were able to disseminate to liver, they failed to spread into spleens of the infected embryos within 48 hpi (Fig. 2B). The ability of PA-0725 to disseminate from the site of inoculation and produce the highest levels of viral transcripts may be related to the shorter survival time of embryos infected with PA-0725.

GVI-PMV1 infection in chicken primary lung epithelial cells

The *in ovo* data demonstrated that there was variation in the replication capacities of the 10 GVI-PMV1 isolates in chicken embryo tissues. We further evaluated replication properties *in vitro* using primary chicken lung epithelial cells. The production of cell-free viral RNA (detected by qRT-PCR) and infectious viral particles (detected by $TCID_{50}$ assay) differed among the 10 GVI-PMV1 viruses at early time points ($P < 0.0001$, ANCOVA; Fig. 3 and S2). At 12 hpi, viral RNA and infectious viral particles were detectable in supernatant of all cultures, with PA-0725 producing the highest amounts. Three viruses from subgroups C and D (PA-0805, na-0101 and PA-0712) had the lowest total amount of cell-free viral RNA at 12 hpi (below 10^8 total viral RNA/ml) but these three viruses had the fastest rate of viral RNA production between 12 and 20 hpi. By 36 hpi all viruses except na-0101 and LaSota had comparable amounts of viral RNA ($\sim 10^{10}$ total viral RNA/ml). All viruses had between 1 and 2 $\log_{10}$ fewer infectious particles than total viral RNA in the supernatant at each measured time point (Fig. S1).

Type I IFN induction by GVI-PMV1 in infected chicken primary lung epithelial cells

Host cells make and release type I interferon (IFN) in response to virus infection. Type I IFNs, also called IFN-α/β, possess pleiotropic capabilities that modulate innate and adaptive immune responses, cell growth, and apoptosis (Goodbourn et al., 2000) and inhibit virus replication. We measured IFN β transcript levels in