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Molecular adaptation of an H7N3 wild duck influenza virus following experimental multiple passages in quail and turkey

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ABSTRACT

To investigate the molecular adaptation of influenza viruses during natural interspecies transmission, we performed a phenotypic and genotypic analysis of a low-pathogenic duck H7N3 influenza virus after experimental passages in turkey and quail. Results obtained showed differences in the HA receptor-binding and in NA enzyme activities in viruses recovered after passages in quail, compared to those obtained from passages in turkey. Sequencing of the HA, NA and genes of internal proteins of the viruses obtained from quail and turkey, identified several amino acid substitutions in comparison with the progenitor virus. Of note, in the quail-adapted viruses the emergence of a 23-amino acid deletion in the stalk of the NA and the introduction of a glycosylation site in the HA were a reminiscence of changes typically observed in nature confirming a potential role of the quail in the adaptation of wild birds viruses to domestic poultry.

Introduction

The history of pandemic influenza A virus infections and the current circulation of a novel pandemic strain in human population stress an intense interest and increasing urgency in understanding viral factors that allow interspecies transmission to human from the natural reservoir of influenza viruses represented by aquatic birds (Horimoto and Kawaoka, 2001; Palese and Shaw, 2007; Smith et al., 2009). To date, it is well known that avian influenza viruses with pandemic potential can be transmitted from avian species to humans through reassortment mechanisms or directly through evolutionary adaptation of viral molecular determinants involved for acquiring a viral tropism for humans (Palese and Shaw, 2007). Avian influenza viruses of the H7, H5, H9 subtypes have been responsible for human infections sometimes associated with fatal cases worldwide (Alexander, 2000; Capua and Alexander, 2004; Maines et al., 2008). However, apart from the scanty indications of the influenza virus transmission to humans directly from the wild bird reservoir (Kurtz et al., 1996), it is hypothesized that terrestrial poultry (chickens, quail, etc.) could act as an intermediate host where viruses from wild waterfowl may acquire mutations that allow their transmission to humans (Gambaryan et al., 2002; Matrosovich et al., 1999; Perez et al., 2003b). Complete understanding of the basis of interspecies transmission of these viruses is an important but yet unrealized goal.

In the last years there has been a sharp increase in the number of outbreaks of avian H7 influenza in poultry, mainly in Europe and Asia (Campitelli et al., 2008). Avian influenza viruses belonging to H7N3 subtype, demonstrating an hemagglutinin (HA) affinity for the human receptor and confirming possibility of infecting of humans pose one of the main fear for human health (Aamir et al., 2009; Belser et al., 2008;

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Morgan et al., 2009; Puzelli et al., 2005). In previous studies a close relationship between H7N3 avian influenza virus isolated in wild waterfowl in 2001 and H7N3 turkeys and chickens viruses subsequently circulating in Northern Italy has been reported (Capua et al., 2002). Circumstantial evidence suggested the direct virus interspecies transmission in turkeys of this H7N3 avian strain circulating in wild waterfowl (Campitelli et al., 2004). Of note, compared to the progenitor obtained from wild duck, the H7N3 viruses isolated in turkeys possessed two amino acid difference in HA and an amino acid deletion as well as specific amino acid changes in the neuraminidase (NA), features which are commonly detected in viruses isolated in terrestrial poultry after direct transmission from wild duck (Banks et al., 2001; Campitelli et al., 2004; Matrosovich et al., 1999). Moreover, it was also shown that the replication in vitro of these turkey viruses differed from that of the progenitor virus circulating in wild duck (Giannecchini et al., 2006). Recently, in order to investigate viral adaptation mechanisms to poultry, it has been reported that, after experimental passages of the wild duck H7N3 influenza virus in quail and in turkey, the quail- and turkey-adapted viruses showed an increased shedding in chicken compared to the original duck strain (Cilloni et al., 2010). Noteworthy, only the quail-adapted virus acquired an increased pathogenicity in chicken. In this study, it was of interest to investigate the molecular adaptation of this duck H7N3 virus after its transmission to turkey and quail. In particular, the virus variants obtained after serial passages of the low-pathogenic H7N3 duck virus in turkeys and quails were subjected to phenotypic and genotypic characterizations.

Results

Characterization of H7N3 influenza viruses used in the study

In order to investigate the molecular adaptation of the well characterized low-pathogenic H7N3 avian influenza virus A/Mallard/IT/33/01 (here thereafter namely Mall/33) after its experimental passages in quail and in turkey (Cilloni et al., 2010), selected viruses obtained in a previous study at different passages and possessing different replicative activity in chicken were chosen to carry out phenotypic and genotypic analysis. Table 1 shows the phenotypic characteristics of the selected viruses after 6 and 10 passages in quail (QA6 and QA10) and after 4 and 10 passages in turkey (TK4 and TK10). All the viruses efficiently replicated on Madin-darby Canine Kidney (MDCK) cells as well as in embryonated chicken eggs (ECE) and possessed similar HA titers (Table 1 and data not shown). It seemed also important to compare the two groups of turkey and quail adapted viruses with regards to their receptor-binding and NA activities. Table 2 shows that all viruses exerted maximal binding affinity for sulphated receptors Su-3'SLN and Su-SLe^x, which is the characteristic feature of H7 poultry viruses (Gambaryan et al., 2008). Moreover, while the original Mall/33 virus had good affinity for the typical duck receptor STF, affinity for this receptor was weakened in all quail and turkey variants with the highest reduction observed for QA10 virus (Table 2). Again, compared to the progenitor Mall/33 strain the QA6 and QA10 viruses increased their binding activity to the human type receptor 6'SLN whereas TK4 and TK10 viruses lost this activity (Table 2). Then the viruses' ability to elute from the cell substrate by using turkey and quail red blood cells (RBCs) was investigated. In this attempt, it was observed that the H7N3 viruses obtained from the quail after 6 and 10 passages were not able to elute from either type of RBCs during an overnight incubation at 37 °C, whereas Mall/33 progenitor virus and the variants obtained from turkey after 4 and 10 passages eluted completely within 2 h (Table 3). None of the viruses eluted in presence of the NA inhibitor oseltamivir, demonstrating that the observed effect was specifically determined by the NA activity. Because this assay reflects the combined effect of HA receptor-binding and NA receptor-destroying activities, we determined the specific viral NA activity using fluorescence-based NA enzyme assay, as previously described (Potier et al., 1979). All selected viruses exhibited similar NA activity compared to the progenitor virus in presence or absence of oseltamivir (Table 3). Altogether these results suggest that during serial passages in quail the H7N3 duck virus acquired a different receptor-binding activity associated with the lack of elution ability from the substrate cells.

Table 1
Passage history and biological characteristic of avian influenza H7N3 viruses used in this study[a].

Virus[b]	History	Replication in animal[c]	$TCDI_{50}$/ml	HA titer with RBC	
				Turkey	Quail
Mall/33	Isolated from wild duck	Low (no)	10^7	256	128
QA6	Mall/33 after 6 passages in quail	Nd[d]	$10^{6.5}$	128	128
QA10	Mall/33 after 10 passages in quail	High (yes)	10^7	256	128
TK4	Mall/33 after 4 passages in turkey	Nd	10^7	256	128
TK10	Mall/33 after 4 passages in turkey	Medium (no)	10^7	128	128

[a] Values are the mean of three independent experiments.
[b] The virus variants are identified with the time of passage and the avian species in which the Mall/33 (A/Mallard/IT/33/01) was inoculated. QA, quail. TK, turkey.
[c] Results obtained by infection of chicken with the indicated quail- and turkey-adapted virus checking viral excretion compared to the progenitor strain. In parenthesis are indicated the presence of clinical signs observed in chickens (Cilloni et al., 2010).
[d] Nd, not done.

Molecular changes in HA and NA glycoproteins of H7N3 turkey- and quail-adapted viruses

In order to investigate the molecular changes of the H7N3 viruses during their passages in turkey and quail, we first sequenced the HA and the NA genes. Fig. 1 shows that the HA of the viruses obtained at selected passages in quail and turkey exhibited amino acid substitutions in 8 positions (4 after the passages of Mall/33 virus in quail and 4 after the passages of Mall/33 virus in turkey). In particular, 6 of the observed mutations (indicated by H7/H3 numbering here thereafter) can be implicated in the viral receptor-binding specificity to the sialic acid receptor (Fig. 2). In fact, V86/96A and E95/105G changes in the QA6 and QA10 viruses are closely located to Y88/98 position which is known to be implicated in the binding to sialic acid receptor. Substitutions T126/136N and S127/137G in the TK2, TK4 and TK6 viruses could affect the hydrogen bonds between the carboxylic group of sialic acid and the side and main chain atoms of amino acids 126/136 and 127/137, respectively. Mutation A151/160T in QA10 viruses introduce a glycosylation sequon at the tip of the HA close to the receptor-binding site. Finally, the substitution G177/186V in the QA10 virus is located in a position well known to be able to affect receptor-binding (Liu et al., 2009; Matrosovich et al., 1997). Conversely, based on their location, the S84/94N and T235/244I changes in the TK4, TK6 and TK10 viruses are not likely to affect the receptor-binding properties. Sequencing of the NA genes of these viruses showed 9 amino acid position changed (F322S and H323C in QA2 variant, V178I, M208I, K212R, I264V and T334K in QA4 variant, I264D and E268D in TK2 variant, Q206P and M208I in TK4 variant; N3 numbering) as well as a 23-amino acid deletion compared to the progenitor virus Mall/33. In particular, the quail adapted viruses acquired the 23-amino acid deletion after 3 passages and maintained it up to 10th passage. The deletion is a typical molecular change observed in viruses of wild aquatic birds after their interspecies transmission and evolution in poultry (Campitelli et al., 2004; Matrosovich et al., 1999). Interestingly, we did not find a deletion in the viruses passaged in turkey. None of the amino acid substitutions observed were present in the turkey viruses