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Creation of diversity in the animal virus world by inter-species and intra-species recombinations: lessons learned from poultry viruses

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Abstract The biological diversity within viruses is one of the largest found in all other forms of nature. Many mechanisms contribute to virus diversity and include incorporating genetic material from the host, recombination between viruses belonging to the same or to a different family, and even recombination between viruses normally infecting different hosts. In particular, avian viruses can utilize all three of these mechanisms to generate new viruses. It is well documented that recombinations can occur between Marek's disease virus (MDV), an oncogenic herpesvirus, fowlpox virus (FPV), and various avian retroviruses. In addition, chicken infectious anemia virus (CIAV), a circovirus, was created by several inter-family recombination events, which occurred between plant and animal viruses. The circovirus represents the ancestral creation of a recombination between a plant DNA virus (nanovirus) and a mammalian RNA virus (calicivirus), through a transition of RNA to DNA made by an endogenous mammalian retrovirus. The present review will discuss the current knowledge on recombination events that have occurred between avian herpesviruses and retroviruses following dual infections in vitro and in vivo. In addition, we will discuss recombinations between fowlpox viruses and the avian retrovirus reticuloendotheliosis (REV). Finally, the review will address the creation of CIAV and how it evolved from recombinations between a plant virus and an animal virus.

Keywords Viruses · Recombination · Herpesviruses · Retroviruses · Circoviruses · Molecular diversity

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Exchanges of genetic material

There is tremendous biological diversity within viruses. This diversity can be primarily attributed to the sophisticated strategies viruses use for recombination.

Exchange of genetic material is a universal mechanism of acquiring variable genomes and functions. Exchanges involve shuffling of genetic material between the host and the infecting virus, between viruses belonging to the same or different families, or between viruses infecting various hosts (Fig. 1). Numerous mechanisms of new gene formation have been described, for either host genes, or viruses. Within higher organisms these processes include atypical splicing, within and between genes, tandem and interspersed segmental duplications, and retrotransposition events [1]. Viruses undergo processes by which they might acquire genomic fragments from the host, a process also denoted as viral piracy [2]. Kim and Kliger [3] reviewed the viral piracy of genes encoding immunomodulatory proteins from the host that helps the virus to evade the host immune system. Members of the Herpesviridae and Poxviridae families are the best-known viruses that have acquired genes encoding homologs of host cytokines, interferon regulatory factors, chemokines, and their receptors. Through these pirated genes, they can mimic or inhibit the effects of both the adaptive and innate immune systems. In one example, the Kaposi sarcoma-associated herpesvirus (KSHV), one of the few human herpesviruses

proved to be associated with tumorogenesis in humans, has extensively pirated cellular cDNA from the host genome [4, 5]. In another study, a Marek's disease virus (MDV) [6] was found in which the entire terminal repeat short segment was replaced with duck genome sequences that corresponded to sequences in the chicken chromosome 19 [7]. MDV also acquired host genes homologous to interleukin-8 (vIL-8) [8] and an RNA telomerase subunit [9].

Chickens have a distinct advantage for the study of virus diversity because they are the natural host and no separate experimental models are needed to perform various studies for which disease reproduction or infections with various pathogens are needed. Therefore, many natural infections of chickens can be considered as reflecting real and natural situations and not artificial events. Moreover, virological studies on poultry have additional advantages because of their relevance to "real life" virology, as the chickens in commercial poultry houses are exposed to environmental stress conditions which might influence the disease outcome. Also, the large number of birds in commercial flocks facilitates the study of low-rate events and multiple infections can be easily reproduced experimentally.

In some of the examples detailed below are molecular recombinations between the five avian oncogenic viruses, that include herpes viruses and retroviruses, all economically important in veterinary virology. We will discuss

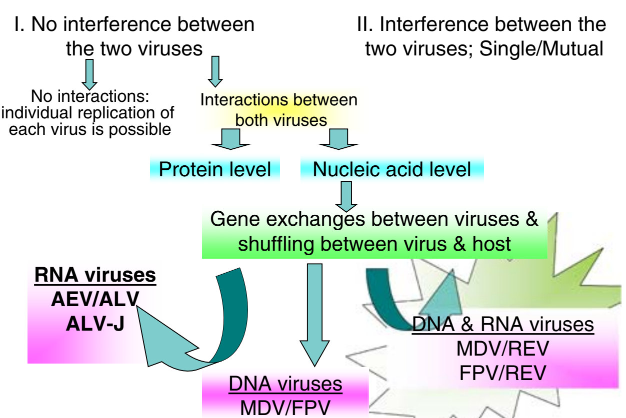

Fig. 1 Schematic description of the various possibilities that can occur in multiple virus-infected animals. In multiple virus-infected cells, viruses can either not interfere with each other or interfere with each other such that one or both viruses do not replicate. In case of no interference, the two viruses can replicate individually in the same cell, without any kind of interaction between them, or they can interact on each other replication, in at least one level, at the protein or genomic levels. In the case that the dual-virus infection of the same cells affects the genome of one virus, a possible outcome would be genomic exchanges between the two viruses. The avian viruses provided a variety of actual examples of shuffling of genomic fragments between RNA viruses, like the spontaneous in vivo creation of ALV-J from the endogenous AEV and exogenous ALV, between DNA viruses, like MDV and FPV, and between DNA and RNA viruses, like MDV and REV and FPV and REV

three situations, naturally-acquired mixed infection, experimentally induced mixed infections in chickens, and multiple virus-infection of embryonated eggs. In a second example of recombination between two virus families, we will discuss fowlpox viruses (FPV), which contain various inserts from reticuloendotheliosis virus (REV). The third example represents a molecular recombination that occurred between viruses of animals and plants which led to the creation of a new family of viruses, the circoviruses.

The avian oncogenic viruses

The avian oncogenic viruses of chickens that we will discuss include one herpesvirus, MDV and three retroviruses; the reticuloendotheliosis virus (REV) [10], avian leucosis viruses subgroups A through E [11], and avian leucosis virus subgroup J (ALV-J) [12].

MDV is a dsDNA avian herpesvirus and is one of the most economically important pathogens that affect the poultry industry. MDV transforms T-lymphocytes, leading to the formation of skin and visceral tumors, but also causes immunosuppression and a variety of symptoms until tumors became visible. MDV is ubiquitous and widely disseminated in poultry flocks worldwide. Natural MDV isolates of a variable virulence have been isolated. Avirulent viruses have been adapted to serve as effective vaccines against virulent MDVs and the prevention of disease. MD comprises a unique example in nature, in that it is the first naturally-occurring malignant disease that is caused by a herpesvirus and can be effectively controlled by vaccination using naturally-isolated avirulent MDVs.

Retrovirus replication within chicken cells employs reverse transcriptase to generate a DNA copy, following which, the viral genome is incorporated into the cellular genome. REV, ALV, ALV-J, and lymphoproliferative disease virus (LPDV) [13] are avian C-type retroviruses. REV transforms pre-B and pre-T lymphocytes, causing bursal and T-cell lymphomas in susceptible chickens and turkeys. ALV transforms B-lymphocytes, causing bursal lymphomas in chickens. ALV-J transforms myeloid cells and causes myelocytomatosis, which are predominantly formed in bones mainly in meat-type broiler breeder flocks. LPDV affects turkeys rarely, and the target cell for transformation is not known.

The tumors are the most prominent clinical sign manifested in poultry following infection with avian oncogenic viruses. The clinical signs caused by infection with the five avian oncogenic viruses overlap and are of a low degree of pathognomy. REV induced T-cell lymphomas are similar both macroscopically and microscopically to those caused by MDV, while REV induced B-cell lymphomas resemble those caused by ALV. Generally, the enlargement of the

peripheral nerves and proventriculus, as well as visceral tumors characterize MDV involvement. The presence of bursal tumors in birds older than 16 weeks is characteristic of lymphoid leukosis while the presence of nerve lesions, bursal tumors, pancreas and intestine involvement suggest REV oncogenesis. The appearance of myeloid tumors in bones is characteristic of ALV-J infections.

Multiple viral infections

In a previous volume of Virus Genes, Becker [14] presented a comprehensive review on the origin and molecular evolution of the genetic diversity of animal viruses. Besides "importing" foreign genes from the host, and evolving from ancient RNA, viruses can exchange genomic fragments with different viruses that co-infect the same cell. In these cases inter-viral recombinations can occur and viruses are able to evolve towards greater molecular and biological diversity.

In some cases of multiple virus infections, the viruses can interfere with each other such as multiple infections with the same ALV subgroups [11]. Conversely, ALVs of different subgroups will not inhibit each other's replication and their dual presence in the same cell could lead to their interaction on various levels, either genomic or protein (Fig. 1). One of the possibilities of genomic interactions is gene exchanges between the viruses that infect the same cell. This topic will be discussed later.

Multiple viral infections are common in poultry. The poultry house environment contains numerous viral, bacterial and fungal pathogens and individual chickens are exposed to a complex cocktail of pathogens. While the multicausal etiology and pathology of respiratory and enteric diseases of poultry are well known [15–17], the present review will concentrate on a group of viral pathogens that cause tumors and immunosuppression in chickens and turkeys.

Beginning in 1993, Israeli commercial flocks were submitted for molecular diagnosis of avian oncogenic viruses and served to evaluate the incidence and the prevalence of flocks and birds that carry multiple viral infections [18–21]. We systematically documented the natural co-infections with MDV and each of the three avian retroviruses (REV, ALV, and ALV-J) [22]. The study included a total of 306 chicken and 59 commercial turkey flocks, of which, about a quarter of the tumor-bearing commercial flocks carried a mixed MDV and retrovirus-infection. A total of 2,926 DNA samples were analyzed, including 2,428 chicken and 498 turkey DNA samples. Of these, 991 DNAs originated from flocks with a multiple virus-infection. Multiple viral sequences were detected by PCR in 103 DNA preparations from that group (103/991—10.4%), including 38 and 56 DNAs from chicken blood and tumor tissues, respectively. Nine turkey blood samples were positive.

The high prevalence (25%) of chicken and turkey flocks with multiple virus infections was surprising (Fig. 3). Multiple virus infections have a double biological significance: (a) in multiple pathogen infections in the same host the clinical and pathological signs might differ from those typical of infection with each pathogen alone; and (b) the presence of multiple pathogens in the same host might lead to genetic interactions between them. The present review focuses on showing that in chicken cells which are dually infected with dsDNA viruses and retroviruses, molecular recombination between the two viruses can occur, leading to increased genetic diversity and to possible changes in biological characteristics.

Although multiple viruses were found in such a high frequency of flocks, their presence in a bird or in a tumor does not necessarily indicate the cause of a particular tumor. The viruses are immunosuppressive and oncogenic by themselves and can jointly influence the deregulation of cellular genes and transactivate each other's regulatory genes. Retrovirus sequences could also modify the original MDV properties by integrating into the MDV genome and altering gene transcription around the insertion site.

Synergism in pathogenicity was observed by several researchers and field veterinarians, however the phenomenon was not well documented. The evident reason for the amplification of the disease severity by a dual virus infection might reside in the lymphotropic nature and the independent transforming and immunosuppressive potentials of each of these viruses. The first report on dual infection of birds with MDV and REV under natural conditions was as early as 1967 [23]. In the following years it was found that several MDV strains influenced both ALV and REV pathogenicity [24, 25]. To assess the in vivo effect of mixed infection with MDV and REV, we documented a doubled mortality rate and decreased bird weight in chickens co-infected with both MDV and REV, compared to single virus-infected groups (Davidson I. unpublished).

Not only was MDV-1 implicated in the increased incidence of retroviral infection symptoms, but serotype 2 MDV infections augmented the development of bursal lymphomas induced by ALV and REV. That phenomenon was not affected by differences in susceptibility or by the immune responses to ALV by the various chicken lines [26–28]. The incidence of spontaneous bursal lymphomas was significantly higher in chickens vaccinated with the bivalent vaccine (SB1+HVT), independent of the genetic background of the birds [29]. The presence of MDV-2 enhanced the hyperplastic follicle formation in the bursa of Fabricius [30–32]. A molecular analysis revealed that

MDV-2 transactivated the ALV LTR promoters in chicken embryo fibroblasts (CEF) [33, 34]. In addition, apoptosis of the retroviral-transformed B cells was inhibited [35].

Molecular recombination between viruses of the same species

Molecular interactions between RNA viruses

Genomic exchanges between viruses can occur between two RNA viruses, between two DNA viruses, or between DNA and RNA viruses (Fig. 1). Among the avian RNA viruses, two retroviruses can recombine either in vitro or in vivo during experimental or native dual virus infections. Recently, in vivo recombination led to the creation of a new avian leukosis virus, subgroup J (ALV-J). ALV-J emerged following a spontaneous recombination between exogenous and endogenous retroviral sequences [36]. The new virus was quickly disseminated worldwide resulting in a devastating economical impact on the poultry industry. Sequencing the ALV-J genome revealed several recombinations had occurred between the gag and pol genes of an exogenous ALV and an endogenous avian retroviral (EAV) sequence [36]. ALV-J represents a viable recombinant that occurred spontaneously in vivo between exogenous and endogenous avian retroviruses. While the LTR, gag and pol genes are highly homologous among other ALV subgroups, the ALV-J env gene has only 40% identity with other exogenous ALV envs, and 75–95% homology with env-like genes of the EAV family [11]. A number of other in vivo recombination events between ALVs have been described: an ALV-J encoding an ALV-A envelope [37, 38]; and an acutely transforming isolate of ALV-J. Recombinant ALVs containing ALV-J sequences are examples of in vivo recombination events occurring between RNA viruses that commonly infect the same lymphocytes in the chicken. It was also recently shown that Australian breeding flocks were co-infected with ALV-A and ALV-J [39].

Molecular interactions between DNA viruses

Multiple viral infections of chickens with DNA viruses are probably the explanation why genetic exchanges occur between these viruses. To our knowledge, two studies documented natural dual infections of chickens with fowlpox virus (FPV), Infectious laryngotracheitis (ILTV) virus [40], and FPV, and MDV [41]. These events might facilitate, in a yet unknown mechanism, transfer of genomic fragments between DNA viruses. Although the rate of these DNA exchanges were believed to be lower than the recombination events which involve RNA viruses, Brunovskis and Velicer [42] provided evidence that MDV contains several FPV homolog genes.

Molecular interactions between DNA and RNA

Herpes and retroviruses

In vitro studies: Integration of the retroviral sequences into the herpesvirus genome was induced in vitro by co-infecting CEF cultures with MDV and either REV or ALV [43–51]. By co-cultivating MDV and REV in the same tissue culture dish Jones et al. [47] created the first recombinant virus, RM1, which was characterized both molecularly and biologically as having an altered in vitro replication and in vivo biological properties [52]. However, co-cultivation of MDV and a retrovirus was not the only mechanism by which retroviruses recombine with MDV. Sakaguchi et al. [53] and Endoh et al. [35] reported retroviral long terminal repeat (LTR) integrations into MDV not as a result of co-cultivation of both viruses, but instead, as a result of culture maintenance or the presence of avian endogenous viruses in the host cells.

Retrovirus integration into MDV occurs because retroviruses can easily integrate into any double stranded (ds) DNA. Integration can occur into the cellular or the dsMDV genome. The documented inserts of avian retroviral sequences, were mainly the LTR, and were located at the junctions between the unique (long or short) MDV fragments and the terminal or internal repeated MDV fragments (TR_L and TR_S and IR_L and IR_S) (reviewed in 50). However, one example was found in which a full length REV genome had been inserted into HVT. The REV was infectious when transfected into CEF cells and presumably could function to produce REV during an HVT infection [45].

In vivo studies: Being able to efficiently generate recombinant viruses in vitro, we asked whether retroviruses could integrate into DNA viruses in vivo as well. If such recombinations occurred, the resulting recombinant MDV might have altered the biological properties. Such recombinants might have altered pathogenicity, virus spread, and antigenic changes that could result in a reduction in the efficacy of vaccinal protection.

We analyzed the in vivo integration events in order eliminate artifacts created by virus replication in vitro. Frequent genetic changes occur upon in vitro virus-replication processes as evidenced by Robinson and Gagnon [54] that showed that a solo LTR was the most common insertion. Jones et al. [47] demonstrated that the retroviral LTR, of the experimentallycreated recombinant virus RM1, undergo duplication during replication in cell

cultures. For that reason we avoided further in vitro replication of the viruses which contained chimeric molecules [22, 55–57]. In spite of difficulties working with viruses in vivo, we showed that retroviruses could integrate into the MDV genome, as exemplified by the detection of chimeric molecules within the DNA that was purified from a tumor-bearing chicken [22, 57].

Unlike in vitro, where recombinant viruses were isolated by several rounds of plaque purifications and limiting dilutions, the in vivo situation differs in that many different events can occur simultaneously in the same bird as each cell produces many herpes virions. As various molecules were formed and detected in the same DNA preparation, recombinant virus isolation was problematic. Only a biological advantage would enable a recombinant virus to dominate in an infected bird.

As the cell culture passage could generate further recombinations, the Hot Spot-combined PCR (HS-cPCR) [58] was developed to amplify recombinant molecules that were present in vivo (Fig. 2). The PCR primers and procedure were based on the LTR inserted in the RM1 virus [47]. Also, the pulse field gel electrophoresis (PFGE) that was used for tissue cultured-MDV separation was inefficient for separating MDV from organs, but was useful with feather tips as a source of the original MDV that was infecting the bird [22, 59]. We concentrated on feathers, because if a recombinant virus would be formed in vivo, its biological significance would be evident by horizontal spread through the feather follicle epithelium [60, 61]. We found that MDV and retrovirus co-infections in vitro and in vivo lead to chimeric viruses with the integration of an

Primer combination	Direct LTR Integration	Inverse LTR Integration
M1+LTR1	No product	M1 3' LTR1 5' 5' 3' LTR
M1+LTR2	M1 5' LTR2 3' 5' 3' LTR	No product
M2+LTR1	LTR1 5' 3' M2 5' 3' LTR	No product
M2+LTR2	No product	LTR2 3' 5' M2 5' 3' LTR

Fig. 2 Schematic description of the HS-cPCR assay, which was designed to detect MDV chimeric molecules that contained retroviral LTR genomic fragments. The amplification employed a heterologous set of primers, one designed to bind to MDV, and the other to bind to the retroviral LTR sequence. The orientation and location of the retroviral LTR insert can be defined by the amplification result of the four combinations of the two primer sets. The insertion site within the MDV genome can be determined by the amplification success following the use of the specific MDV primers (forward or reverse, marked 1 or 2) and the size of the amplicon. The insert orientation can be determined by the amplification success using the direct or the reverse retroviral LTR primer (marked 1 or 2)

LTR into the MDV genome. The chimeras appeared in low quantities and as quasispecies that interfered with sequence analysis of cloned gel-purified DNA.

In addition, the in vivo herpes-retro recombination differs and is more complex than in vitro recombination. The cells in the in vitro co-infection were chicken embryo fibroblasts, whereas in vivo, the co-infected cells are mostly lymphocytes and monocytes. While the recombination rate in vitro was rather high, the in vivo formation of viable recombinant viruses was lower. Nevertheless, we showed that infection of commercial poultry can result in the emergence of recombinant viruses that could possibly have unexpected biological properties.

Retroviral integration into herpesviruses in vivo has significance in general virology and veterinary medicine and also represents a special case of gene transposition. To determine the occurrence of such integrations in vivo, we studied and followed these chimeric molecules. Several conditions were analyzed: (a) commercial birds that acquired a natural mixed infection; (b) experimentally co-infection of chickens with prototype strains of MDV and ALV-J; and (c) commercial chickens infected experimentally with virus obtained from commercial cases of double infection with MDV and ALV-J. In the two first cases, we found that integration events happened at different rates, depending on the experimental system used. While in commercial flocks the recombination event was limited (about 2.5% of the 2,926 DNA samples), it reached a 30–50% rate in experimentally-infected birds, and was undetectable in experimentally-infected birds using field isolates. It appeared that by increasing the virus adaptation to laboratory conditions, the rate of retrovirus LTR integration into MDV increased, as judged by the percent of birds with chimeric molecules.

Using the HS-cPCR assay, we found that about 5% of the total blood and tumor tissue DNAs, prepared from birds with a double MDV and retrovirus infection, contained chimeric molecules (Fig. 3). In actuality, the rate may be even higher, as the HS-cPCR method is selective for relatively short chimeric molecules and is designed to detect insertion sites that are proximal to the MDV primer sites that were shown in vitro to serve as the hot spots for LTR integration [42].

For the first time, we demonstrated that both in vitro and in vivo co-infections with MDV and each of the three avian retroviruses (REV, ALV and ALV-J) can lead to retroviral LTR integration into MDV [22]. Although we were not able to determine whether viable viruses were generated, we were able to demonstrate the presence of a variety of chimeric molecules and map the integration sites that occurred in the birds. The PCR amplification of the REV- and the ALV-LTR insertions in the chimeric molecules demonstrated that a large fragment of LTR had been

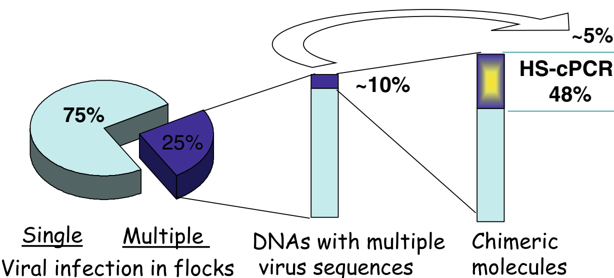

Fig. 3 Schematic representation of the percentage of chimeric molecules in double virus-infected birds in commercial flocks. About 25% of the Israeli commercial flocks that were analyzed contained multiple virus-infections of MDV and one of the retroviruses. About 10% the total DNA samples that originated from these flocks, contained both MDV and retroviral sequences. We detected chimeric molecules in about half of the DNA samples from multiple virus-infected tissues. Overall, the rate of chimeric DNA molecules in relationship to the total DNA samples with multiple virus-sequences was about 5%

inserted into MDV. That finding also supported the idea that these integrations were recent and might be a direct consequence of a bird co-infected with the two viruses. These inserts were not only the traces of ancestral LTR integration into MDV, as shown earlier by Isfort et al. [44] to be represented by the presence of short (20 bp) LTR stretches with a 70% homology or more into various MDV strains, but most probably they indicate recent recombination events that occurred in the mixed-infected bird.

In each DNA preparation, a variety of chimeric molecule types were detected, indicating the in vivo formation of molecular quasispecies in dually-infected birds. The chimeric molecule heterogeneity we found may indicate that several integrations occurred in one double virus-infected cell. Since the DNA preparations originated from multiple cells, our findings may also indicate that recombination has occurred in several cells. The diversity of viral quasispecies in a host might also result from vaccination selective pressures [62, 63].

Another implication of this study concerns recombination events between MDV, or avirulent MDV vaccine strains and endogenous retroviruses. About 5–7% of the mammalian and human genome was found to be comprised of endogenous retroviruses [48, 64, 65]. A similar feature was reported for many endogenous retroviruses residing in the chicken genome [66], and these are also liable to integrate their LTRs into the MDV genome.

Recently, Cui et al. [67] demonstrated the spontaneous creation of MDV and retrovirus chimeras in Chinese commercial flocks. Chimeric viruses had spread to commercial chicken flocks. The recombinant viruses were re-isolated in tissue culture and shown to contain retroviral LTR inserts of 540 bp. These chimeras apparently caused a more severe thymus and bursa of Fabricius atrophy than was expected [67].

Fowlpox and avian retroviruses

Pox is a viral disease of commercial poultry (chickens and turkeys), as well as of pet and wild birds (reviewed in 68). Fowlpox caused by the fowl pox virus (FPV) is economically a significant disease because it causes a drop in egg production and increased mortality. However, pox usually has a mild clinical appearance and spreads at a slow rate, causing discrete nodular proliferative skin lesions on the non-feathered parts of the body (cutaneous form) or fibrino-necrotic and proliferative lesions of the mucous membrane of the upper respiratory tract, mouth, and esophagus (diphtheric form). A simultaneous systemic infection might appear in some cases. Disease prevention is achieved by vaccination with live FPV or antigenically similar pigeon pox strains produced either in chicken embryo fibroblasts (CEF) or on chorioallantoic membranes of chicken embryonated eggs (CAM) [68].

In recent years, infections with FPV reappeared in poultry flocks in several countries [69–74] and the poor efficacy of vaccines against the new emerging isolates has generated a renewed interest. The recent events of FPV-like mortality raise additional concerns: (a) the disease and its prevention by the presently available vaccines. It is important to determine whether FPV isolates with novel characteristics emerged lately, and whether the presently available vaccines are effective in face of the presence of new FPV isolates, (b) the introduction of REV infection through FPV as a "Trojan horse" vehicle for the dissemination of REV. Molecular insertion of retroviruses into large DNA viruses leads to the creation of chimeric viruses, and this phenomenon is an emerging danger, regarding both the new FPV isolates and the spread of REV. Solo LTR insertions, with their associated promoter and enhancer activities, can alter adjacent gene transcription levels.

The evolving story of retrovirus infection associated with FPV began in 1973 in Israel, when Bendheim [75] reported cases of a neoplastic disease in turkeys after FPV vaccination in the years 1966–1969. A 25–34% mortality was observed in experimentally-infected chickens due to the REV infection that caused enlargement of the visceral organs and lymphoid cell infiltrations. A contamination of an FPV vaccine with REV occurred in the US and was identified by Fadly et al. [76]. Subsequently, various methods used to screen fowlpox vaccines for contamination with REV have been described [77, 78].

REV integration into large DNA viruses was documented in both the virulent and vaccine strains of fowlpox virus. These findings were documented in the USA, Germany, Australia, and also lately in Israel (Davidson, unpublished) [73, 79–82]. Some FPV strains appear to contain full-length copies of the REV genome, while others

contained REV-LTR fragments of various lengths. In one case, transfection of cells with FPV containing a full-length REV resulted in the production of infectious REV, infection of chickens and seroconversion [80]. A REV-LTR remnant sequence was detected in the Australian FPV M, while a full length REV provirus was detected in the FPV S vaccine strain and several Australian field isolates [80]. Similar to MDV, the LTR was found to be the most stable integrated fragment. It paralleled the findings of Robinson and Gagnon [54] that showed that large parts of integrated proviruses could be lost from host DNA over time, whereas the LTRs remained. Singh et al. [74] documented the mechanisms of retrovirus genome exclusion from the FPV genome. Various FPV isolates contained heterogenous REV genomic fragments, ranging from nearly intact REV provirus to just 248 or 508 bp REV-LTR fragments, all inserted at the same genomic site.

Unlike MDV, where the retroviral-LTR integrations occurred primarily at sites within the junctions of the unique and repeated regions of MDV, the retroviral-LTR integrations occurred within the FPV genome at a specific site between open reading frame 201 and 203 [50, 82, 83] analyzed early isolates of FPV and showed that the original integration of REV into FPV occurred more than 50 years ago. However, REV envelope sequences have been detected only in FPV field strains, suggesting the presence of intact REV genome in wild-type strains. It is not understood whether the emergence of new FPV isolates and the apparent lack of vaccine efficacy are due to the REV insertions. It is also not known whether other avian retroviruses, like ALV, ALV-J or LPDV (in turkeys) have integrate/ed into FPV.

Circoviruses derive from a Molecular recombination between plant and animal viruses

The origin of circoviruses represents an unusual genetic transfer from plants to vertebrates. A plant virus belonging to the nanovirus family probably recombined with a vertebrate-infecting calicivirus virus, an RNA virus. A retrovirus or retrotransposon-mediated reverse transcription may have facilitated the recombination that generated circoviruses. Circoviruses are circular, single-stranded small DNA viruses with host specificity for bacteria, animals, humans, and plants. The avian circovirus, chicken infectious anemia virus (CIAV), induces both symptomatic and asymptomatic disease, primarily causing immunosuppression. The mechanisms of circoviruses pathology are poorly understood.

Molecular similarities in the replication initiator protein (Rep) of circoviruses suggest a link to both the small plant viruses belonging to the nanovirus family and to small RNA viruses in animals, such as caliciviruses [84]. A series of recombination events probably led to the creation of circoviruses, a new virus species. The initial interspecies recombinations occurred in the Rep protein, which initiates the rolling circle replication at the nonanucleotide sequence within the origin of replication (*Ori*). Extensive genomic alignments and phylogenetic analysis of the Rep proteins revealed that the circoviruses diverged from a plant virus ancestor. The N-terminus of circovirus and nanovirus Reps are homologous from the N-terminus to position 129.

The C-terminus of the circovirus Rep protein was found to be related to the animal calicivirus and other picorna-like RNA virus 2C-proteins. Picorna-like viruses code for a polyprotein that is cleaved proteolitically to produce the 2C protein, an RNA-binding protein that is homologous to the C-terminal part of the circovirus Rep protein. The Rep protein binds specifically to the DNA, cleaves and ligates DNA at conserved sequences within the *Ori*. In both circovirus and nanovirus DNA, the *Ori* is adjacent to the N-terminal part of the Rep gene and suggests their common ancestral origin and function. Consequently, Gibbs and Weiller [84] concluded that following the host-switching event of the nanovirus transfer from plant to animal, the sequence survived because it was complete and could replicate. The Rep and *Ori* activities were essential and replication proceeded using the host cell DNA polymerase. Additional recombinations could have occurred, enhancing the enzymatic activities of the new virus.

Another recombination event probably occurred in the C-terminal region of the circovirus Rep protein. Since the calcivirus is an animal virus, this recombination is believed to have happened in the vertebrate cell itself. As Caliciviruses are RNA viruses and do not have reverse transcriptase activity, whereas circoviruses are DNA viruses, it was hypothesized that a retrovirus or a retrotransposon must have contributed a reverse transcriptase for the recombination to take place.

Summary

Recombination between retroviruses and herpesviruses can occur at a detectable frequency. In a broader context, this is potentially significant because the generation of viral chimeras probably plays a role in the generation of new pathogens and in the evolution of novel viruses. The issue of retroviral sequence integration into herpes viruses in vivo, in cases of double virus-infection is of a wide significance in general virology and veterinary medicine and also represents a special case of gene transposition. Using the avian model, we sought to determine how frequently such in vivo integrations resulted in chimeric viruses.

In addition to retroviral interactions with DNA viruses, large DNA viruses have acquired cellular homologs from their hosts. FPV and MDV share a putative ORF for a common protein [42]. Dual infections can occur in the same bird, and the resulting recombinations can be viewed as a "natural genetic engineering" of new viruses. These recombinations can also occur in human viruses. The chicken is an ideal animal model for studying these natural recombinations. In addition, emerging viruses and molecular dynamics are of a great veterinary importance in terms of poultry health and economic parameters.

Chickens provide a unique study model that cannot be reproduced experimentally in other in vivo systems. The advantage of commercial chicken flocks consists of the fact that these viruses cause natural infections in the natural host. Moreover, the stress and environmental conditions are natural and not artificial. Also, the available populations for research are large, thus allowing the study of rare events. As a consequence of a multiple viral infection, molecular recombination between DNA viruses and retroviruses might lead to the emergence of new MDVs with altered properties, including tropism, shedding pattern, pathogenicity, and vaccinal protection.

Since most human populations are infected with some of the eight human herpes viruses (HSV1, HSV2, VZV, CMV, EBV, HHV6, HHV7, and HHV8) and may also be dually infected with retroviruses, like the human immunodeficiency virus (HIV), the present study could provide an animal model to understand the consequences of dual infections in humans. One example of co-infection in humans involves Kaposi sarcoma, where both HHV8 and HIV have been implicated in the development of the disease. In addition, HHV6 and HIV were proposed as co-factors for the development of the acquired immunodeficiency syndrome (AIDS). In another study HHV6 and HHV7 were implicated as major opportunistic viral infections in AIDS patients. Based upon the ease of generating avian herpes virus–retrovirus chimeras in chickens, research should be initiated to determine whether retrovirus–human herpes virus chimeras can develop in humans and if so, the possible consequences of such chimeras in human health.

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