Viral Evolution

12

Contents

Viruses are ideal objects for studying evolutionary processes because of their short generation time, high numbers of offspring that they produce during infection and not least because of their simple structure. Viruses must continuously adapt to the conditions of their host or their host populations, so selection mechanisms are accessible to experimental approaches. In this context, different criteria play an important role, such as the antigenic diversity, the extent of virus excretion, and the degree of virulence. The complete adaptation of a virus to its host, which leads to a minimization of virulence of the infectious agent, is for both parties the desirable consequence: i.e. a problem-free coexistence and survival. For example, hepatitis G virus (GB virus C) which was initially isolated from patients with liver inflammation, seems to persist in many people without causing illnesses. A similar situation is observed with torque teno viruses (► Sects. 14.5 and ► 20.2). Spumaviruses are also found in many animal species and humans without causing symptomatic infections (► Sect. 18.1). For many viruses, the maximum exploitation of genetic variability is not always useful. Viruses reach a limit at which a greater variance is no longer advantageous: the proportion of non-infectious virus variants among the progeny becomes too high, whereby the potentially possible error limit is reached.

12.1 How Do Mutations Lead to the Emergence of Novel Viruses?

The members of the various virus families use different replication strategies. Consequently, the evolution of all viruses differs. For the replication of their

S. Modrow et al., *Molecular Virology*, DOI 10.1007/978-3-642-20718-1_12,

genome, RNA viruses are reliant on the activity of viral RNA-dependent RNA polymerases, which do not have exonucleolytic proofreading activity. This 3′–5′ exonuclease activity is associated with cellular DNA polymerases and verifies the correct addition of the corresponding complementary nucleotide at the 3′ end of an elongating DNA chain during the synthesis of a new DNA strand. If a mismatching nucleotide is erroneously incorporated, the subsequent polymerization step is transiently blocked, and the mispairing nucleotide will be excised by the associated proofreading 3′–5′ exonuclease activity. This error-avoidance mechanism essentially contributes to the high accuracy of cellular DNA replication, which has an average error rate of 10^{-9}; i.e. one erroneous mispairing nucleotide per billion synthesized nucleotides. Therefore, many more mismatched nucleotides are found in most RNA viruses, which exhibit an error rate of 10^{-3}–10^{-4}. Assuming that a virus with an intact genome has a length of about 10,000 nucleotides, the genomes of progeny viruses will differ from the genome of the parent virus by one to ten nucleotides. In fact, one is not dealing with a uniform virus population but, strictly speaking, with a population of very closely related viruses, a phenomenon that is referred to as the concept of quasispecies formation. This is known especially for hepatitis C virus and human immunodeficiency virus (HIV) (▶ Sects. 14.5 and ▶ 18.1). Basically, the same also applies to DNA viruses, in which the phenomenon is considerably less pronounced, as the mutation rate is lower by a factor of at least 10^2. Polyomaviruses, papillomaviruses and parvoviruses (▶ Sects. 19.2, ▶ 19.3 and ▶ 20.1) use cellular DNA polymerases for replication of their genetic information, and are accordingly genetically much stabler than RNA viruses. By contrast, the complex DNA viruses such as herpesviruses and poxviruses (▶ Sects. 19.5 and ▶ 19.6) have their own DNA polymerases with 3′–5′ exonuclease activity for checking for and correcting erroneously incorporated nucleotides. Therefore, the generation of quasispecies does not play a major role in these viruses.

Besides the selection of individual virus variants due to mutations that confer on them a selective advantage in the offspring generation, sometimes there is a simultaneous selection of independent mutations, which are per se not advantageous for the emerging viruses, but accidentally arise as hitchhiking (passenger) mutations together with other beneficial mutations. This situation is particularly complex in viruses whose genes are encoded by overlapping reading frames, as is the case in hepatitis B virus (▶ Sect. 19.1). Permanently evolving viruses are real consequences of all these evolutionary processes. If the mutations are associated with changes of pathogenic properties, they can have decisive consequences for the infection process in their hosts and their survival.

The emergence of “successful” new viruses is based on two independent mechanisms as well as combinations of them:

1. The genetic alteration (mutation) of a virus and its selection
2. The change of the social structures and/or the living and environmental conditions of the host population.

Mutations of the viral genetic information can be manifested in various ways. The consequence of mutations in the genes that code for viral surface proteins, and

thus are subject to selection pressure by the immune system, is called antigenic drift. This antigenic drift is especially pronounced in RNA viruses, such as the caliciviruses, orthomyxoviruses and retroviruses. It is associated with the formation of quasispecies and has a considerable pathogenetic significance (▶ Sects. 14.2, ▶ 16.3 and ▶ 18.1). Like the selection pressure of the immune system on viral surface proteins, antiviral chemotherapy can also exert a selection pressure on mutations in polymerase genes, for example, leading to the formation of therapy-resistant virus variants (▶ Sect. 9.2).

Furthermore, a permanent emergence of new viruses which arise independently of the selection pressure of the immune system or antiviral chemotherapy is also observed. A well-studied example is canine parvovirus, which arose from the pathogen of feline panleucopenia by only a few mutations (▶ Sect. 20.1.6). Canine parvovirus emerged initially in dog populations in Europe in 1978, and was disseminated to all continents in a few months during a pandemic (▶ Chap. 11). That pandemic was associated with high mortality, so millions of dogs died of the haemorrhagic gastroenteritis caused by the virus. Today, it is known that mutations in the genome of the well-known feline panleucopenia virus are responsible for the emergence of canine parvovirus. Only three amino acid substitutions in the capsid protein of feline panleucopenia virus were sufficient for the emergence of the canine virus with an altered host tropism. These mutations alter the receptor binding site of the virus, and enable the new pathogen to bind to canine cells. Pedigree analysis of different viral genomes provided evidence that canine parvovirus did not emerge directly from feline panleucopenia virus, but that arose from feline panleucopenia virus via infection of wild carnivores, especially European red foxes.

Moreover, there is good evidence that the great diversity of picornaviruses (▶ Sect. 14.1) has emerged from a common ancestor virus and has evolved by point mutations during evolution. Because of the high error rate of the viral RNA-dependent RNA polymerase, whose activity is essential for these RNA viruses, mutations arise during viral genome replication with the above-mentioned rate of 10^{-3}–10^{-4}. In addition, recombination processes also play an important role in the generation of new RNA viruses and many other pathogens (Sect. 12.2).

12.2 How Do Viruses Gain New Genes and Functions?

In addition to mutations, viruses with segmented genomes, such as orthomyxoviruses, bunyaviruses, arenaviruses, birnaviruses and reoviruses (▶ Chaps. 16 and ▶ 17), can also undergo profound genetic changes, known as genetic reassortment. This refers to the exchange of one or more genome segments between two related viruses which have infected a cell simultaneously. If the genetic redistribution leads to exchange of genome segments that encode viral envelope proteins, the newly emerging viruses gain a new antigenic pattern, which is called antigenic shift. Well-documented examples of this are the classic pandemics which were triggered in the last century by influenza A virus. Under the names Spanish flu,

Asian flu and Hong Kong flu, they have influenced world history and caused millions of fatalities (▶ Sect. 16.3). The pandemic viruses usually represent genetic reassortants of human and avian influenza A virus subtypes. Swine infected with both subtypes are a "mixing jar" for the emergence of novel influenza viruses. Productive double infections can arise in them, as they are susceptible not only to porcine influenza viruses, but also to avian and human influenza viruses. In contrast, avian influenza A virus subtypes can infect humans generally only in exceptional cases by very close contact, as poultry is not susceptible to human influenza virus subtypes. These relationships confer on porcine influenza A virus infections a specific and potential zoonotic significance, since they can transmit new virus variants to humans.

Besides the known generation of reassortants in influenza A viruses, this process is also known among reoviruses. The ubiquitous occurrence of rotaviruses in calves and piglets and the potential threat of genetic reassortment with human strains put these infections under a particular spotlight (▶ Sect. 17.2).

Nevertheless, even viruses with non-segmented genomes are able to interchange large gene regions. This mechanism is known as genetic recombination. It is enabled by changing the use of the template strand during nucleic acid synthesis, a process that may occur when certain cells of an organism are infected with two different but related virus types. Genetic recombination has been described in a variety of viruses and is particularly documented in different RNA viruses. Classic examples are togaviruses from the group of the New World equine encephalitis viruses (▶ Sect. 14.6). Western equine encephalitis virus, which causes acute encephalitis in horses and humans, originated by genetic recombination between eastern equine encephalitis virus and a Sindbis-virus-like isolate. Today, Sindbis virus is detectable only in the Old World; however, a similar virus must originally have coexisted with eastern equine encephalitis virus on the American continent before it was displaced by the newly emerged western equine encephalitis virus.

Genetic recombination is possible not only between two related viruses, but also between viral and cellular nucleic acid molecules – a process that often has great pathogenetic importance. Well-studied examples are the oncogenic retroviruses, which have incorporated a cellular oncogene in their genetic information, and thus are able to generate tumours in their hosts (▶ Chap. 18). Avian Rous sarcoma virus and feline sarcoma virus are examples.

Not only cellular oncogenes have been integrated into viral genomes, genetic recombination events have also been described with other cellular genes. For example, bovine diarrhoea virus, a flavivirus, has integrated the ubiquitin gene in its RNA genome by recombination with cellular messenger RNA during evolution. This gene has been incorporated in the region that encodes the viral non-structural proteins. In this way, a new cleavage site is created within the polyprotein, which is recognized by the cellular ubiquitin hydrolase. This process is associated with an alteration of the phenotype: starting from an originally non-cytopathic virus, a pathogen with high virulence has emerged which causes a fatal disease in chronically infected cattle, which is known as mucosal disease (▶ Sect. 14.2).

12.3 What New Infectious Agents Have Emerged Recently?

The origin of HIV-1 and HIV-2 can be explained by repeated host changes (different monkey species in West Africa) and their transmission to humans. In Africa, monkeys are infected with different species-specific variants of simian immunodeficiency virus (SIV); however, they do not become sick. HIV-1 probably originated from a chimpanzee virus (SIVcpz) that was transmitted to humans (▶ Sect. 18.1.5). In contrast, HIV-2 has developed from a type of SIV found in sooty mangabeys (SIVsmm) which has been repeatedly transmitted from monkeys to humans. During an adaptation phase in the new host, humans, the virulence was increased and virus variants were selected that were able to spread effectively from person to person. The pandemic-like character of HIV infections could be developed only by a radical change in living conditions and social structures in Africa. Trade, urbanization and global tourism have to be mentioned in this context. Since the emergence of HIV-1, different subtypes have arisen by mutations, and these have different geographical distributions. These processes are additionally influenced by genetic recombination between the different subtypes. Epidemiologically important in this context is that the type of HIV-1 (HIV-1B) that is found in Europe and in North America differs from the types that are found in Africa (HIV-1A, HIV-1C) in the efficiency of their transmissibility: HIV-1B specifically infects cells in the intestinal mucosa, which is considered as a result of the selection of transmission by homosexual practices.

Chikungunya virus, a togavirus that is transmitted by mosquitoes, causes a highly febrile human disease in tropical countries, in southeastern Africa and on the Indian subcontinent (▶ Sect. 14.6). In 2007, Chikungunya virus infections were observed for the first time in northern Italy because the tiger mosquito has become endemic in southern Europe and the virus found its way to Europe through infected mosquitoes or patients. If this pathogen can adapt to other European native mosquito species by mutations in the coming years, then this tropical infectious disease will possibly spread also in central Europe. Toscana virus (▶ Sect. 16.2), which is transmitted by sandflies, is another pathogen that causes meningitis and feverish diseases, and might also spread from southern to northern Europe because of global warming.

Bluetongue virus, a ruminant pathogenic virus (cattle, sheep, goat, deer), is ubiquitous in Africa and in some regions of southern Europe. In 2006, bluetongue virus (serotype 8) was described in Germany for the first time. Starting from a first outbreak in Belgium, bluetongue virus spread swiftly by infected culicoids (midges). At the end of 2007, the Benelux countries and Germany were extensively affected. In addition, there were also outbreaks in France and the UK. It is not known how the virus was introduced. Similarly, the role of global warming on viral development in arthropod hosts is also not clear.

SARS virus was first described as a clinically very severe pneumonia in Southeast Asia in the autumn of 2002 (▶ Sect. 14.8). Presumably, it was transmitted by contact of people with certain species of civet cats, which are traded in markets, and then spread to the human population very fast. Some rigorous national and

international measures have been taken to control the outbreak of this per se animal pathogenic virus, and to prevent its adaptation to humans as a host organism. Similarly, it is feared that the widespread and highly pathogenic avian flu virus H5N1 may change by continuous mutations and adapt to humans as its preferred host (▶ Sect. 16.3). Initially it infected only poultry in Southeast Asia, but now also populations of migratory birds are affected, and these have transported and spread this highly pathogenic virus strain from Asia to Europe and Africa. However, in a few exceptional cases people can be infected who do not transmit the virus further. Nevertheless, there is concern that the virus could change by mutations during its reproduction in infected patients. This may create new variants of the H5N1 virus which would be adapted better to humans, spread in the human population and might lead to the emergence of a novel very dangerous influenza pandemic. Therefore, drastic measures have also been taken in this case, e.g. culling of infected livestock when the first suspected cases occur, in order to control the emergence of the H5N1 virus in commercial poultry and to minimize the risk of transmission to humans. Unlike H5N1 virus, the H1N1 influenza A virus subtype, which caused flu in humans for the first time in Mexico in April 2009, exhibits a relatively low pathogenicity. Therefore, Mexican flu spread worldwide within a few months and became a new pandemic. The development of a new reassortant in swine is considered as causal for the emergence of the novel subtype.

Further examples of new zoonotic transmissions are Nipah virus and Hendra virus in Southeast Asia and Australia. Dramatically changed living conditions in the habitat of the host, fruit bats (flying foxes, *Pteroptus giganteus*), caused by human interventions in the form of massive land clearing and deforestation also plays a crucial role. This forced the bats to find new habitats, namely habitats shared with swine (Nipah virus) and horses (Hendra virus). Swine and horses may be infected via pasture or feed contaminated with the saliva of infected bats. From these species the viruses found their way to humans in a second step.

The occurrence of bovine spongiform encephalopathy is an example of the impact of industrialization processes in agriculture on the emergence of a new infectious disease. By feeding cattle, which are physiologically genuine herbivores, with inadequately inactivated animal proteins originating from animal waste of sheep and cattle carcasses, the heat-stable agent was transmitted to this species and caused the emergence of bovine spongiform encephalopathy, an animal and human pathogenetically and economically (variant Creutzfeldt–Jakob disease in humans) highly significant disease.

Therefore, considering the ubiquitous and fast processes that occur in the evolution of viruses, special caution is required. Factors that can promote and support the accidental emergence of a new virus should be avoided whenever possible. These include the imprudent use of live vaccines, particularly those that can establish persistent infections in the vaccinees, and especially the use of viruses as biological weapons to reduce or eradicate certain hosts, as occurred during the control of the rabbit plague in Australia by administration of rabbit haemorrhagic disease virus and myxoma virus (▶ Sects. 14.8 and ▶ 19.6). The exposure of naive populations of potential hosts to a novel virus can cause a non-calculable biological

disaster, just as we have seen in the infections with the new canine parvovirus (► Sect. 20.1). Of course, the use of viruses as a weapon for bioterrorism must be prohibited as well. Last but not least, such use of pathogenic agents can contribute, in addition to catastrophic pandemics, to the emergence of new virus types, which would also have fatal effects on their developers and users.

Further Reading

Chevillon C, Briant L, Renaud F, Devaux C (2008) The Chikungunya threat: an ecological and evolutionary perspective. Trends Microbiol 16:80–88

Domingo E, Gomez J (2007) Quasispecies and its impact on viral hepatitis. Virus Res 127:131–150

Domingo E, Webster R, Holland J (1999) Origin and evolution of viruses. Academic, San Diego

Duffy S, Shackelton LA, Holmes EC (2008) Rates of evolutionary change in viruses: patterns and determinants. Nat Rev Genet 9:267–276

Goudsmit J (1997) Viral sex – the nature of AIDS. Oxford University Press, Oxford

Kay A, Zoulim F (2007) Hepatitis B virus genetic variability and evolution. Virus Res 127:164–176

Lemey P, Rambaut A, Pybus OG (2006) HIV evolutionary dynamics within and among hosts. AIDS Rev 8:125–140

Peiris JS, de Jong MD, Guan Y (2007) Avian influenza virus (H5N1): a threat to human health. Clin Microbiol Rev 20:243–267

Wong S, Lau S, Woo P, Yuen KY (2007) Bats as a continuing source of emerging infections in humans. Rev Med Virol 17:67–91