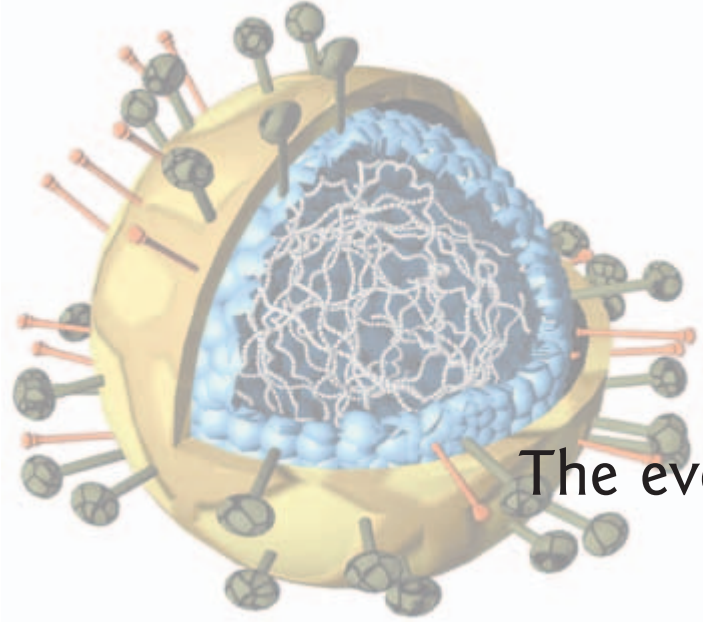

17

The evolution of viruses

Like all living organisms, viruses are not static entities but are subject to evolutionary pressures and undergo evolutionary change.

Chapter 17 Outline

Viral genomes accumulate mutations in the same way as all other nucleic acids and, where conditions enable a mutant to multiply at a rate faster than its fellows, that mutant virus will have an advantage and will succeed the parental type. Where viruses have an evolutionary advantage is that many polymerases (notably in the RNA viruses) lack proofreading mechanisms, so that mistakes in replication are not corrected. Thus, mutants accumulate more rapidly than seen with DNA-based organisms where DNA replication has proofreading. Viruses also have a shorter generation time relative to their host. In any discussion of evolution, it is pertinent to ask where viruses first came from (see Section 1.9). The absence of any fossil records and the scarcity of other evidence have not, of course, prevented scientists from speculating about their origins. The two prevailing opinions are that viruses have either arisen (i) from degenerate cells that have lost the ability for independent life, and/or (ii) from escaped fragments of cellular nucleic acid.

The history of viruses extends as far back as man's historical written or pictorial records. Amongst the earliest references is a 3500-year-old bas relief sculpture from Egypt depicting a man supporting himself with a crutch. Careful examination shows that the calf muscle of his right leg is withered in the characteristic aftermath of paralytic poliomyelitis. A slightly later excavation of the same civilization found the mummified body of the pharaoh, Rameses V, whose face bears the characteristic pock marks of smallpox. Such evidence tells us of the long association of these viruses with mankind.

Whatever their origins, viruses have been a great biological success, for no group of organisms has escaped their attentions as potential hosts. Viruses are largely species-specific with respect to their host and usually do not cross species boundaries. It seems likely that every animal species has the same range of viruses as occurs in humans. In Chapters 13–16, the various ways in which viruses interact with their hosts are discussed, illustrating how viruses cause a variety of changes, ranging from death to the imperceptible. Evolution of any successful parasite has to ensure that the host also survives. The various possible virus–host interactions can be thought of as different ways in which viruses have solved this problem.

17.1 THE POTENTIAL FOR RAPID EVOLUTION IN RNA VIRUSES: QUASISPECIES AND RAPID EVOLUTION

The lack of a proofreading function in the polymerases of RNA viruses means that base substitutions (mutations) occur at the rate of between 10^{-3} and 10^{-5} per base per genome replication event (i.e. one mutation for every 1000–100,000 newly synthesized bases). Not all of the resulting mutants will be viable but many are, resulting in an extremely heterogeneous population of viruses. This is called a quasispecies. This has a number of implications. For example, it is not possible to define the genome sequence of that virus population precisely, and any sequence in a database will represent only one member of the quasispecies. The quasispecies phenomenon also endows RNA viruses with the ability to adapt rapidly to and to exploit any environment they occupy. This is seen on both a micro and a macro level, and arises because one or more members of the quasispecies will inevitably have a selective advantage over others. The micro level is infection of the individual, and virus evolution during the life time of the individual is seen, particularly in life-long infections with HIV-1 (see Chapter 19) and hepatitis C virus (see Section 20.8). It is likely that in these infections, the immune system is a major selective force, and that the quasispecies phenomenon allows these viruses to persist in the face of that immune response. The macro level of evolution is seen in viruses with a worldwide distribution. This could impinge upon people with different genetic backgrounds, especially those that affect the MHC (HLA) haplotype that controls T cell immunity. This leads to the establishment of different specific genetic sequences, known as genotypes, or clades of a virus in different parts of the world, and a classification that is based on sequence rather than serology (e.g. HIV-1, Chapter 19).

Influenza A virus does not cause persistent infections but virus variants evolve under the selection pressure of antibody stimulated during

an acute infection. These variants are in turn passed on to susceptible individuals in a country or a continent. Thus, the virus evolves with the effect that, in approximately 4 years, it is not recognized by the immune memory established by the original host, and he or she can be reinfected (Section 17.5). This is called *antigenic drift*, and essentially results in the formation of new serotypes of virus.

Not all RNA viruses exhibit such obvious variation, even though they have the same potential for change as HIV-1 and influenza virus. For example, the overall antigenicity of polioviruses types 1, 2, and 3, and measles virus has not changed over their known history of approximately 50 years, and the original vaccines still provide the same level of protection. Thus, by comparison with HIV-1 and influenza virus, the polioviruses and measles virus are stable. However, their genomes do accumulate mutations, and minor changes in antigenicity (in individual epitopes) of the virion can be detected by probing with monoclonal antibodies. In addition, viruses from different global areas can be distinguished by their genotype. It is not understood why, for example, measles virus is antigenically stable and influenza virus is not, as in many ways these two infections are similar (both viruses are highly infectious, spread by the respiratory route, and cause acute infections). Presumably measles virus is under a less effective or more constrained evolutionary selection pressure than influenza virus, but exactly what these pressures are is not known.

17.2 RAPID EVOLUTION: RECOMBINATION

Recombination is the other major force in virus evolution and takes place in a cell that is simultaneously infected by two viruses. Usually, the two genomes are highly related with regions of homology between their genomes that permit the replicating enzyme to move from one strand to another. Thus at a stroke, the daughter molecule has some of the properties of both parents. However, both parts of the resulting genome have to be sufficiently compatible for the progeny to be functional. Both DNA and RNA genomes undergo recombination. Detailed monitoring of the HIV-1 pandemic shows new recombinant viruses arising between different clades, and such plasticity makes the prospect of control by vaccine problematic (see Chapter 19). Viruses with segmented genomes can also undergo recombination by acquiring an entire genome segment from another virus, and this occurs at higher frequency. This form of recombination is known as *reassortment*. The effect on antigenicity is enormous as a virus can acquire an entirely new coat protein in a single step. The prime example is influenza A virus where this process, called *antigenic shift*, is responsible for pandemic influenza in man (Section 17.5). However, a viable recombinant requires compatibility

between all eight genome segments, and this puts some limitation on the creation of new viruses.

17.3 EVOLUTION OF MEASLES VIRUS

Measles virus

- *Member of the paramyxovirus family.*
- *Infects only humans.*
- *Unusual in that every infection causes disease.*
- *Infection results in lifelong immunity.*
- *Measles can be countered by use of a live vaccine.*

F. L. Black studied the occurrence of the measles in island populations (Table 17.1), and found a good correlation between the size of the population and the number of cases of measles recorded on the island throughout the year. A population of at least 500,000 is required to provide sufficient susceptible individuals (i.e. new births) to maintain the virus in the population. Below that level, the virus eventually dies out, until it is reintroduced from an outside source.

On the geological time-scale, humans have appeared only recently and have only existed in population groups of over 500,000 for a few thousand years. In the days of very small population groups, measles virus could not have existed in its present form. It may have had another strategy of infection, such as persistence, which would have allowed it to infect the occasional susceptible passer-by, but there is no evidence of this.

Table 17.1

Correlation of the occurrence of measles on islands with the size of the island population. (From Black, F. L. 1966. *Journal of Theoretical Biology* **11**, 207–211).

Island group	Population ($\times 10^{-3}$)	New births per year ($\times 10^{-3}$)	Months of the year in which measles occurred (%)
Hawaii	550	16.7	100
Fiji	346	13.4	64
Solomon	110	4.1	32
Tonga	57	2.0	12
Cook	16	0.7	6
Nauru	3.5	0.17	5
Falkland	2.5	0.04	0

However, Black has speculated upon the antigenic similarity of measles, canine distemper, and rinderpest viruses. The latter infect dogs and cattle respectively, animals that have been commensal with humans since their nomadic days. Black suggests that these three viruses have a common ancestor which infected prehistoric dogs or cattle. The ancestral virus evolved to the modern measles virus when changes in the social behavior of humans gave rise to populations large enough to maintain the infection. The first such population occurred 6000 years ago when the river valley civilizations of the Tigris and Euphrates were established.

17.4 EVOLUTION OF MYXOMA VIRUS

Myxoma virus

- *Member of the poxvirus family.*
- *Natural host is the South American rabbit in which it causes only minor skin outgrowths.*
- *Also infects the European rabbit causing myxomatosis, with lesions over the head and body surface; infection is 99% lethal.*
- *Is spread by arthropod vectors that passively carry virus on their mouth parts: mosquitoes in Australia and rabbit fleas in the UK.*

Myxoma virus was released in England and Australia upon wholly susceptible host populations of the European rabbit in an attempt to control this serious agricultural pest. This experiment in nature was carefully studied with respect to the changes occurring in the virus and the host populations and provides an object lesson in the problems of biological control.

In the first attempts to spread the disease in Australia, myxoma virus-infected rabbits were released in the wild but, despite the virulence of the virus and the presence of susceptible hosts, the virus died out. It was then realized that this was due to the scarcity of mosquito vectors, whose incidence is seasonal. When infected animals were released at the peak of the mosquito season, an epidemic of myxomatosis followed. Over the next 2 years, the virus spread 3000 miles across Australia and across the sea to Tasmania. However, during this period it became apparent that fewer rabbits were dying from the disease than at the start of the epidemic. The investigators then compared the virulence of the original virus with virus newly isolated from wild rabbits by inoculating standard laboratory rabbits. Two significant facts emerged: (i) rabbits infected with new virus isolates took longer to die, and (ii) a greater number of rabbits recovered from infection. From this it was inferred that the virus had evolved to a less virulent form (Box 17.1). The explanation was simple:

Box 17.1

Evidence for the evolution of myxoma virus to avirulence in the European rabbit after introduction of virulent virus into Australia in 1950

After release into Australia viruses were isolated from wild rabbits and their virulence tested by infecting laboratory rabbits. The percentage of survial in the laboratory rabbits and the mean survival times were calculated.

Mean rabbit survival time (days)	Mortality (%)	Year of isolation			
		1950–1951	1952–1953	1955–1956	1963–1964
<13	>99	100	4	0	0
14–16	95–99		13	3	0
17–28	70–95		74	55	59
29–50	50–70		9	25	31
>50	<50		0	17	9

mutation produced virus variants which did not kill the rabbit as quickly as the parental virus. This meant that the rabbits infected with the mutant virus were available to be bitten by mosquitoes for a longer period of time than rabbits infected with the original virulent strain. Hence the mutants could be transmitted to a greater number of rabbits. In other words, there was a strong selection pressure in favor of less virulent mutants which survived in the host in a transmissible form for as long as possible.

The second finding concerned the rabbits themselves, and the possibility that rabbits were evolving resistance to myxomatosis. To test this hypothesis, a breeding program was set up in the laboratory. Rabbits were infected and survivors were mated and bred with other survivors. Offspring were then infected, the survivors mated and so on. Part of each litter was tested for its ability to resist infection with a standard strain of myxoma virus. The result confirmed that the survivors of each generation progressively increased in resistance. However the genetic and immunological basis for this is not understood.

This work shows (i) how a virus which is avirulent and well adapted to peaceful coexistence with its host can cause lethal infection in a new host, and (ii) how evolutionary pressures rapidly set up a balance between the virus and its new host which ensures that both continue to flourish. The latter remains a stumbling-block for biological control of pests that attack animals or plants. Today, rabbits are still a serious problem to agriculture in the UK and Australia.

17.5 EVOLUTION OF INFLUENZA VIRUS

Influenza A viruses

- *Members of the orthomyxovirus family.*
- *Comprise 144 viruses (subtypes) that naturally infect aquatic birds; cause subclinical gut infections and virus is spread by the oral–fecal route.*
- *Can evolve to infect man, via domesticated poultry; causes respiratory disease that is spread by infected droplets; serious morbidity and mortality, although can be subclinical.*
- *Initial introduction of a new influenza virus in man is called an* antigenic shift*; virus then undergoes gradual change or* antigenic drift *– Darwinian evolution in response to positive pressure from virus-specific antibody. Shift and drift refer to changes in the two major surface proteins of the virus particle.*
- *Influenza can be countered by use of a killed vaccine, and recently by a live vaccine, and by antiviral drugs.*

Background

There are three groups of influenza virus, types A, B, and C, that all cause respiratory disease in man and can be distinguished by the antigenicity of their internal virion nucleoproteins. Type A viruses cause the worldwide epidemics (pandemics) of influenza, and both type A and B viruses cause epidemics. Type C viruses cause only minor upper respiratory illness and will not be discussed further. In terms of natural history, the primary hosts of influenza A viruses are wild aquatic birds (such as ducks, terns, and shore birds). Influenza B viruses infect only humans. In man A and B viruses cause disease only in the winter, usually January and February in the Northern hemisphere, and June and July in the Southern hemisphere. At the equator, virus is present at a low level throughout the year. The cause of this periodicity is not known. In addition, a limited number of other species are naturally infected with type A influenza viruses. These and the directions of transmission are shown in Fig. 17.1. More recently (2005), cats and dogs were found for the first time to be infected.

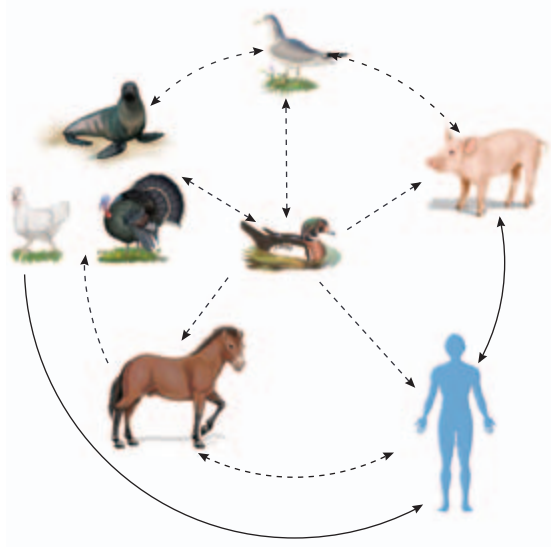

Fig. 17.1 Animal species that are naturally infected with influenza A viruses. Wild birds of the sea and shore form the natural reservoir (top). Known routes of transmission are indicated by continuous arrows and probable routes of transmission by broken arrows.

Typical influenza in man is a lower respiratory infection with fever and muscular aches and pains, but can range from the subclinical to pneumonia (where the lungs fill with fluid). In the elderly and people

Box 17.2

Influenza viruses have a formal descriptive nomenclature

Influenza virus type A

Subtype (example) H3N2
Where H is the hemagglutinin and N is the neuraminidase. There may be any permutation of H subtypes 1–16 and N subtypes 1–9.

Strain (example) A/HongKong/1/68(H3N2)
The strain designation indicates (in order): the type, where the strain was isolated, the isolate number, the year of isolation, the subtype.

The nomenclature of nonhuman strains also includes the host species, e.g. A/chicken/Rostock/1/34(H7N1).

with underlying clinical problems of the heart, lungs, and kidneys, and in diabetics and the immunosuppressed, influenza can be life-threatening. Immunity to an influenza virus is effective at preventing reinfection by that same strain. The viral antigens relevant to protective immunity are the envelope glycoproteins, the hemagglutinin (HA) and neuraminidase (NA) (see Figs 3.14 and 3.15), and immunity is mediated by virus-specific antibody. Despite the induction of the immune response repeated infection with influenza virus is common. The repeated infections are possible because the HAs and NAs of influenza A and B viruses evolve continuously so that previously acquired immunity is rendered ineffective. The processes involved in the evolution are discussed below.

Influenza A viruses are classified formally as *types*, *subtypes*, and *strains* (Box 17.2). There are 16 subtypes of hemagglutinin and nine subtypes of neuraminidase. All 144 permutations are found in nature in wild aquatic birds. By comparison, influenza in man is a side show, with currently only two subtypes, H1N1 and H3N2, in circulation.

Two mechanisms of evolution

Influenza A viruses undergo two types of change affecting their major surface glycoproteins called *antigenic shift* and *antigenic drift* (Box 17.3). Since the start of modern virology there have been four shifts that occurred in 1889, 1918, 1957, and 1968. A shift results in a pandemic (a worldwide epidemic), is always preceded by an abrupt change in hemagglutinin subtype and, apart from the 1968 virus, also by a change in neuraminidase subtype. Drift results in epidemics, and is caused by gradual evolution under the positive selection pressure of neutralizing antibody. A new shift virus immediately starts to undergo continuous antigenic drift.

Box 17.3

Evidence for antigenic drift and shift in influenza virus

- Antigenic drift causes epidemics of influenza A and B in man. It results from a gradual change in antigenicity of the influenza virus particle hemagglutinin (HA) and neuraminidase (NA) proteins, through amino acid changes that affect antibody epitopes (see Figs 3.14 and 3.15). It takes ≥4 amino acid substitutions in ≥2 antigenic sites, and approximately 4 years, to evolve a drift variant that can escape immunity previously acquired to the parent virus and so cause significant disease.
- Antigenic shift causes influenza pandemics in man. Only influenza A viruses are involved. Shift results from an influenza virus acquiring a new HA and usually new NA. This can arise by genetic reassortment of an existing human virus with a bird virus that provides the new HA and NA genes. It may also arise by a bird virus adapting/evolving to grow in man, and may involve intermediary mammalian hosts. Once formed, a shift virus causes a sudden pandemic that spreads around the world in 1–2 years, but it takes an unknown time (years?) to evolve to this point. A new shift virus starts drifting immediately.

Influenza B viruses only undergo drift and this is believed to be because they infect only man and have no other animal reservoir.

The complex evolutionary processes that influenza viruses undergo can be better understood against a background of their biology. These viruses are highly infectious and cause an acutely cytopathogenic infection. Thus, they are a victim of their own success which results in a near universal immunity among hosts who, given adequate conditions, can live for around 80 years. Apparently, the production of immunologically naïve individuals by new births is not enough to allow influenza A and B viruses to survive. Thus, they have evolved strategies for changing their antigens in order to increase the proportion of susceptible individuals in the population.

The phenomena of antigenic shift and drift in influenza have significant implications for human infection and they are monitored by a worldwide network of laboratories, coordinated by the World Health Organization (WHO), that isolate and classify currently circulating influenza viruses. In this way new strains can be quickly spotted and the vaccine changed appropriately.

Antigenic shift

The appearance of shift viruses in man is shown chronologically in Fig. 17.2. Virus was first cultivated in the laboratory in 1933 by intranasal inoculation of ferrets with some nasal secretion from a virologist (Wilson

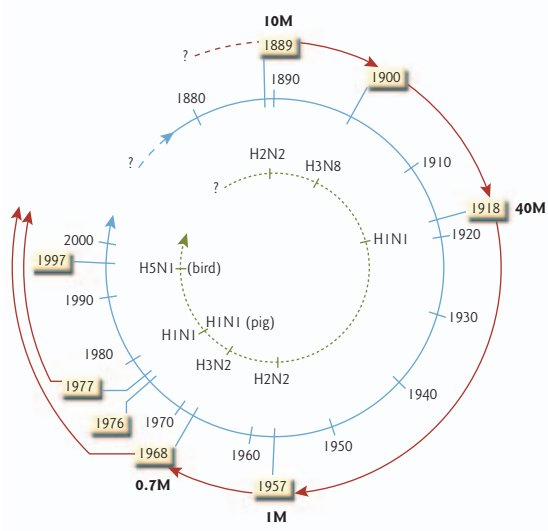

Fig. 17.2 History of antigenic shifts of influenza A viruses in humans. The outer circle denotes the year of emergence of a new subtype that is shown in the inner circle, the duration of the reign of that subtype, and when it is replaced by another subtype. A time scale is shown in the middle circle. Approximate worldwide mortality figures for each shift are indicated in millions. The 1900 shift did not cause a serious pandemic. Currently (2006) H3N2 and H1N1 subtypes coexist. Some occasional infections of people with bird or pig viruses that are not transmitted person to person are noted in the inner circle (but see text).

Smith) who had influenza, and was then successfully passaged in embryonated chickens eggs. However, retrospective information has also been obtained by studying influenza virus-specific antibodies in human sera that had been kept stored in hospital freezers for other purposes. As can be seen, antigenic shift has occurred sporadically from the first recorded shift of 1889 at intervals of 11, 18, 39, 11, and more than 32 years. A brief history of the terrible 1918 pandemic is shown in Box 17.4. Shift, by definition, introduces a virus into a population that has no pre-existing immunity, so it is always associated with an explosive pandemic with high morbidity and mortality, although these vary between different shifts. Since 2001, a new H1N2 reassortant virus has been isolated in many countries in mainly young people. However, this virus has not spread widely, presumably because it is sensitive to existing immunity to its parent human H1N1 and human H3N2 viruses. Approximate mortality figures are included in Fig. 17.2.

Until 1977, only one virus subtype was in circulation at any one time, and this virus was replaced completely when a new subtype was introduced. What causes the original subtype to disappear is not known. In 1977, during the reign of the H3N2 subtype, an H1N1 virus appeared that was identical to the H1N1 virus that had been in circulation in 1950. Thus this was not strictly speaking a shift, but a reintroduction. Where the 1977 virus came from is not known. It had not been infecting the human population during its "absence" as it would have undergone 27 years of antigenic drift (see below). It is as if it had been frozen, and some say that this is literally what happened. However, this is conjecture. At the time of writing, the drifted descendants of the 1968 H3N2 and the 1977 H1N1 cocirculate and continue to undergo antigenic drift. However a new shift could occur at any time.

The mechanism of antigenic shift

As indicated, the natural reservoir of influenza A viruses comprises wild aquatic birds, such as ducks, terns, and shore birds. These avian viruses are tropic for the gut (not the respiratory tract), cause a subclinical infection, and are evolutionarily stable. Many of these birds migrate enormous

Box 17.4

1918 influenza

The 1918 shift virus was unusual in several ways. It is estimated that in just 1 year it killed around 40 million people worldwide. (The enormity of this can be appreciated when compared to the 34 million that have died in 25 years of the AIDS pandemic.) The highest mortality was in young adults and not the elderly and other high risk groups. The overall mortality rate was 25 times greater than other pandemics (2.5% compared with 0.1%). Neither the virulence of the 1918 virus nor its age tropism is fully understood, and it is feared another high mortality virus might arise at any time.

The seemingly intractable problem of analyzing the virus from 1918 that had never been cultivated was solved by extracting fragments of virus genome from the preserved tissue of victims of that pandemic. These are either pieces of lung preserved in formalin in the US army pathology archives or from the exhumed bodies of people who died and were buried in the Arctic permafrost. Virus RNA has been analyzed by RT-PCR and and the complete genome has now been sequenced. Phylogenetics allows hypothetical family trees of influenza A viruses to be constructed, but the resulting relationship of the 1918 virus with other viruses is perplexing. The analysis of the sequence suggests that the virus adapted rapidly from birds to man and was not a reassortant.

Reverse genetics has allowed the functions of 1918 RNAs to be investigated by making infectious virus which has one or more of its RNAs replaced by a 1918 virus RNA. The main finding is that the 1918 HA converts an avirulent virus into one that is pathogenic for mice. This reassortant virus stimulates macrophages to make cytokines and chemokines that attract inflammatory cells into the lung and causes local hemorrhage – as did the virus in people in 1918. Exactly how the 1918 HA differs in this regard from other HAs is not clear.

A complete infectious 1918 virus, A/Brevig Mission/1918, has now been recreated in the USA using reverse genetics and viral RNA from a body preserved in the permafrost. This has caused immense concern worldwide as nobody in the world has immunity to the 1918 virus, and the consequences of an escape would be horrific. However, it was considered that the need to understand what made the virus so terrible made the risk worth bearing. A raft of safety measures including a high level containment laboratory were used for this experiment. Compared with an H3N2 virus, also derived by reverse genetics, the 1918 virus needed no added protease to cleave the HA and form infectious virus, grew to higher titer in mouse lung, caused more lung pathology, and killed chicken embryos. The H3N2 virus with the 1918 HA had increased virulence, but was still less virulent than the complete 1918 virus, showing that other 1918 genes also contribute to virulence. In short, the reconstructed 1918 virus is highly virulent. However, what makes it virulent is still not understood.

distances (e.g. from Siberia to Australia) and spread virus as they go via infected feces. In essence, antigenic shift in the circulation of human influenza virus occurs when virus from a wild bird infects a human being (Fig. 17.3). However, like most viruses, avian influenza virus is species

Box 17.5

Some major evolutionary groupings of vertebrates

Class *Reptilia*: reptiles
Class *Aves*: birds
Class *Mammalia*: monotremes (e.g. platypus)
marsupials (e.g. kangaroo)
placentals (e.g. man)

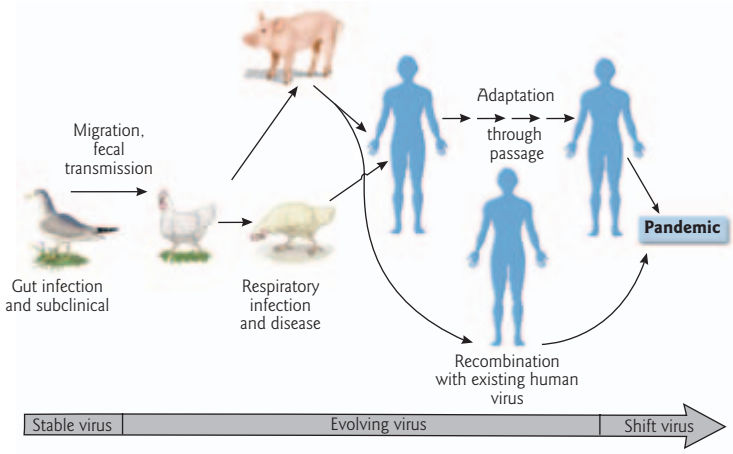

Fig. 17.3 Summary of the events leading up to an antigenic shift of human influenza virus. The evolutionary time scale is not known but probably takes several years.

specific and does not readily infect other bird species let alone mammals which are members of another major taxonomic group (Box 17.5). Thus, the virus has to adapt by progressive mutation before the jump to humans can occur.

It is thought that the first link in the chain is the infection of freerange domestic poultry (mainly chickens, ducks, turkeys, and quail) by migrating wild birds. This is an avirulent respiratory infection, although it is not understood how the switch from a gut to respiratory infection comes about. The infection passes from bird to bird until mutations take place in the protease cleavage site of the hemagglutinin precursor polypeptide. Cleavage is necessary for virus infectivity. Normally, the HA can only be cleaved by a protease that is present solely in the respiratory tract, but after an increase in the number of basic amino acid residues in the HA cleavage site it can be cleaved by another protease that is widely distributed throughout tissues, and this allows the virus to grow throughout the body and cause serious disease. At this stage the virus is able to jump from Class *Aves* to Class *Mammalia*, and multiply (but not yet spread) in humans (Fig. 17.3). One of the major gaps in modern virology is the understanding of animal virus transmission in general, and what gene or genes are responsible for its control. Another variation in the evolutionary progression of virus from wild birds to humans may involve domestic pigs.

In rural areas poultry and pigs are often kept together, giving ample opportunity for the crucial bird-to-mammal adaptation. At this point the virus has two evolutionary options. It can continue to accumulate

mutations and become better adapted to humans, or it can recombine with a human influenza virus strain and so acquire genes that are already fully adapted. In fact, it may do both. One essential adaptation is a change to the viral attachment site on the HA protein so that the virus can use as a receptor an *N*-acetyl neuraminic acid that has an α2,6 linkage to the carbohydrate moiety on a glycoprotein or glycolipid (as is found in cells of the human respiratory tract), rather than the α2,3 linkage that is most common in birds. The advantage to the virus of achieving this by recombination is that at its simplest, there need only be a recombination of the RNA segment that encodes the new hemagglutinin with the seven existing segments from the human strain that control person-to-person spread. This scenario would account for the 1968 shift where an H3N2 virus replaced the H2N2 virus.

Recombination in influenza viruses is very efficient as the genome is segmented – it comprises eight single-stranded, negative-sense RNAs. The hemagglutinin and neuraminidase proteins are encoded by distinct RNA segments. When a cell is infected simultaneously with more than one strain of virus, newly synthesized RNA segments reassort at random to the progeny (Fig. 17.4). Many, though not necessarily all, of the 2^8 (256) possible genetic permutations that can be formed between two viruses are genetically stable. Reassortment occurs readily in cell culture, in experimental animals, and in natural human infections between all type A influenza viruses, but not between A and B viruses. Even after a reassortment event, more mutational adaptation may be needed before the virus is able to cause a recognized pandemic. Thus it may be that a shift virus is present in the human population, undergoing mutational adaptations for a few years before it causes a pandemic.

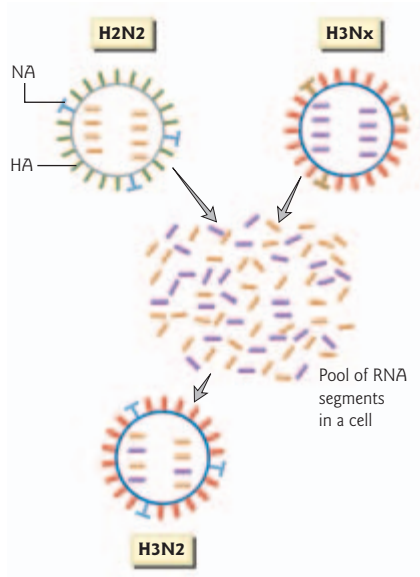

Fig. 17.4 Recombination (reassortment) between an existing human influenza A virus (H2N2) and a new virus from the wild bird reservoir (H3N*x*, where *x* represents an unknown neuraminidase subtype; see text) that gives rise to antigenic shift. The two viruses simultaneously infect a cell in the respiratory tract, and the eight genome segments from each parent assort independently to progeny virions. The example shows a novel progeny virion (H3N2) that comprises the RNA segment encoding the H3 avian hemagglutinin and the seven remaining segments from the existing human virus.

Tracking influenza viruses in domesticated animals

Two instances of abortive antigenic shifts took place in the eastern USA in 1976 and in Hong Kong in 1997. These viruses were noted and notable because they caused death in a small number of young people, and it was feared that they signalled the start of a new lethal pandemic. Both had their origins in nonhuman animals. The 1976 virus was also isolated from local domestic pigs, and the 1997 H5N1 virus from chickens that had been brought as live birds to the local poultry market. Of 18 people infected with H5N1 virus, six (33%) died, but these represent deaths among hospitalized, seriously ill cases, and it is not known

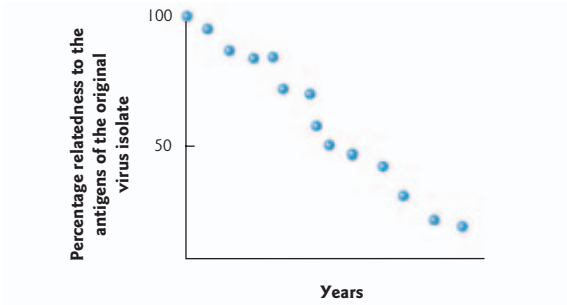

Fig. 17.5 Diagram showing antigenic drift of type A influenza virus in humans. This could represent either the HA or NA. Each point is a virus strain isolated in a different year.

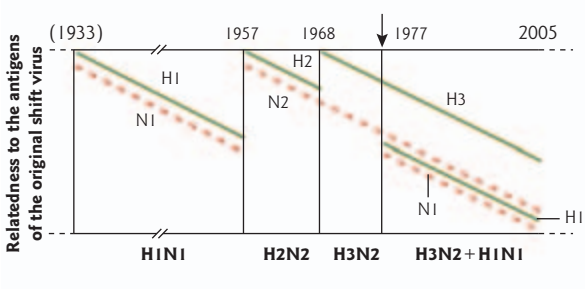

Fig. 17.6 Diagram showing the course of antigenic shift and drift of influenza A viruses in man. The first virus, isolated in 1933, was H1N1. This arose by antigenic drift from the 1918 virus. Other shift viruses appeared in 1957 (H2N2) and 1968 (H3N2). A 1950 H1N1 virus reappeared in 1977. Drift is shown schematically. The 1957 N2 was acquired by the H3N2 shift virus, and has drifted from 1957 to the present day.

how many mild cases there were. However, neither virus had the ability to spread from person to person, and no more infections were seen. Since that time, surveillance has increased, particularly in southeast Asia, of domestic poultry and infected people in order to get an early warning of any impending pandemic. The H5N1 virus was isolated only from poultry in 2001 and 2002, but then caused some cases of fatal disease in humans in 2003, 2004, and 2005. It has been isolated from countries in southeast Asia including Indonesia, Thailand, Vietnam, Japan, South Korea, Cambodia, Laos, and China. Human infection rates are low but mortality has been put as high as 67%. Usually there is H5N1 infection of people's own chickens, and presumptive bird-to-human transmission. There may have been very occasional human-to-human transmission but, in the absence of a pandemic, this is clearly (and fortunately) still an inefficient process. Outbreaks in poultry cause alarm and have provoked culls of 20 million or so birds by the authorities. Other economic costs are also huge as trading partners ban imports of poultry products. The known species range of influenza A viruses was extended in 2003 when H5N1 virus spread with some fatalities to tigers and leopards in a Thai zoo, as a result of animals being inadvertently fed infected chicken carcases, and domestic cats have been infected experimentally. In the last few years various subtypes have been isolated from poultry and humans, including H9N2 virus which causes only mild respiratory disease in man in China and Hong Kong, H7N7 virus in Holland and Belgium (with one human fatality), H7N1 in Italy, H7N2 virus in the USA, and H7N3 in Canada; viruses isolated from pigs in Europe and North America include H1N1, H1N2, and H3N2 subtypes. In 2005, H3N8 virus was found for the first time in dogs in the USA. This overall increase in virus isolation may partly be the result of better surveillance rather than any rise in virus dissemination. To avoid the possibility of serious infection, workers handling infected birds wear safety suits and are offered prophylactic anti-influenza drugs.

Antigenic drift

Influenza A viruses have been isolated every year from humans around the world since their discovery in 1933. Each new isolate is tested serologically with antibody to all other influenza strains. It soon became apparent that the hemagglutinin and neuraminidase of new isolates were antigenically slightly different from those of the previous year, and that the difference increased incrementally year by year. This difference is reflected in one or more amino acid substitutions in one or more of the antigenic sites on the HA or NA. This phenomenon is explained by the assumption that influenza virus mutants carrying modified antigenic determinants have an evolutionary advantage over the parent virus in the face of the existing immune response. However, although both the HA and NA drift, the HA is the major neutralization antigen and therefore appears to be evolutionarily the more significant. Thus year-by-year new amino acid substitutions – and new epitopes – appear. Epitopes can also disappear if a mutation creates an attachment site for a carbohydrate moiety that then sterically prevents antibodies from reacting with those epitopes. The name "antigenic drift" is very apt (Fig. 17.5). The nature of the selective force which drives this process is discussed below. In practice a drift variant that can cause significant disease arises about every 4 years, and this has on average four or more amino acid substitutions in two or more antigenic sites. At the same time the "old" strain is completely replaced by the "new" strain. Influenza B viruses also undergo antigenic drift, but this is a slower process and also several different strains cocirculate. The reason for this difference is not known.

Antigenic drift is at least as important in causing human influenza as antigenic shift. In the UK there are 4000–14,000 deaths associated with epidemics of influenza every year, and this extrapolates to 400,000 to 1,400,000 deaths per year worldwide. Thus, in the twentieth century, drift viruses were responsible for approximately 40 to 140 million deaths worldwide. (In the UK an influenza epidemic is formally defined as 400 cases of influenza per 100,000 people.)

Fig. 17.7 Model of antigenic drift of influenza type A and type B viruses. Points on the same level represent drift variants that arise in the same year. The branch length indicates the relative change in antigenicity from virus in the preceding year. Drift is shown for an arbitrary 7-year period. See text for further discussion.

The mechanism of antigenic drift

As soon as a new shift virus appears and infects people, it begins to drift (Fig. 17.6). Drift of influenza A viruses is linear due to the dominating effects of favorable mutations (Fig. 17.7). Influenza B viruses undergo less drift than type A viruses, and multiple virus lineages coexist. Drift happens on a global scale. It can only be theorized how drift takes place as it is an assumption that drift variants arise from virus circulating in the previous year. It is generally believed that variants are selected by neutralizing antibody (Box 17.6).

Box 17.6

Testing antigenic drift in the laboratory

- Antigenic drift has been modeled in the laboratory using a neutralizing monoclonal antibody (Mab) specific for the HA. Virus and MAb are mixed together and inoculated into an embryonated chicken's egg. This is a surprisingly efficient process and, after just one passage, the progeny virus is no longer neutralized by the selecting MAb. This "drift" virus is also known as an *escape mutant*, and represents the growth to population dominance of an antigenic variant virus that already existed in the inoculum. Sequencing shows there is a single amino acid substitution in the expected antigenic site. If the drift virus is subjected to a MAb to a different epitope, then the process repeats and the progeny virus now has two amino acid changes compared with the original (Fig. 17.8). However if two or more MAbs are mixed together to resemble an antiserum, no progeny virus – mutant or wild-type – is produced at all. Thus drift can only take place if an antiserum effectively contains antibody to only a single epitope.
- Influenza virus HA molecules have five antigenic sites (although sometimes one is hidden by a carbohydrate group). In H3 viruses these are labeled A–E. Each site comprises around 10 epitopes, thus there are 50 epitopes in all, and in theory during infection the immune system should make antibodies to all of these. However when tested by immunizing rabbits with virus and measuring the amount of antibody made to individual epitopes, it was found that one epitope in site B was dominant, and there were only traces of antibody to two other epitopes (Fig. 17.9). The amounts varied between animals. In mice the results were similar but the dominant epitope was in site A. Thus for unknown reasons the immune system responds selectively to foreign epitopes.
- While some antisera were completely neutralizing, others were shown to select escape mutants like a MAb. Thus it would seem that drift operates because certain individuals have an antibody response that is highly biased to one epitope. The derivation of the ≥4 amino acid substitutions in ≥2 sites, referred to above, could therefore be achieved as shown in Fig. 17.10. The biased antibody response may be genetically controlled, and drift variants may arise in a subpopulation with the relevant antibody response. Further, since adults suffer repeated influenza infections, they are likely to make a complex antibody response that cannot give rise to drift variants. The simplest antibody response is likely to occur in children after their first infection, and this may be responsible for the selection of drift variants.

Unsolved problems relating to influenza virus

Despite considerable knowledge of influenza virus (the complete genome sequence, the atomic structure of the HA protein, infectious nucleic acid) there are major areas of ignorance. Most of these concern viral epidemiology

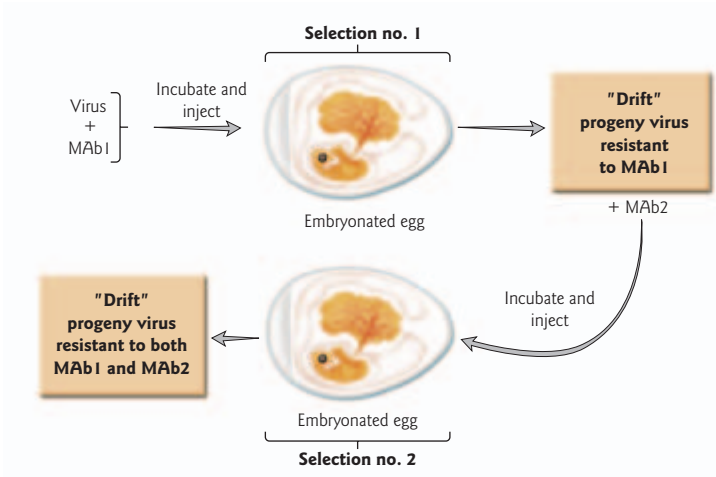

Fig. 17.8 "Antigenic drift" in the laboratory: a single neutralizing monoclonal antibody can select a population of influenza virus escape mutants that is no longer neutralized by the selecting MAb. Another round of selection with a second MAb produces virus that now carries two amino acid substitutions, but selection with the two MAbs simultaneously is completely neutralizing (not shown).

which is immensely difficult to study. Space precludes discussion but the following problems still require an answer about an important though common virus:

- Why is influenza a winter disease?
- How does the same virus appear simultaneously in different places around the globe at the start of an epidemic (i.e. it does not appear to follow any apparent transmission chain)?
- Why is the seasonal restriction of influenza apparently unaffected by the year-round introduction of virus by air travellers incubating influenza? Does this virus not infect people in summer or does it infect them without causing disease?
- How does a clinically significant drift variant travel from northern to southern latitudes in 6 months (and did so prior to frequent air travel)?
- How does a shift virus (other than the reoccurring H1N1 of 1977) replace the existing virus?
- How does a drift virus replace the existing virus?
- The hypothesis of antigenic drift outlined above needs verifying.
- Why did the 1918 shift virus have a higher mortality than other shift viruses?

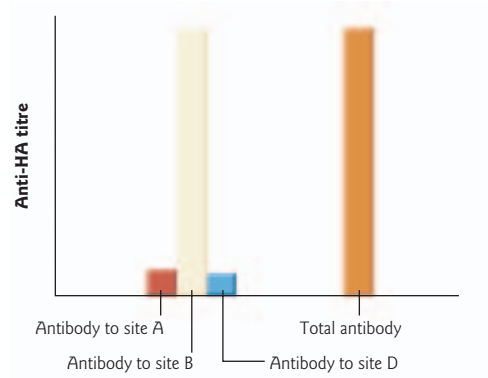

Fig. 17.9 An epitope-biased serum antibody response in a rabbit injected with influenza A virus. Nearly all the HA-specific antibody is accounted for by the response to a single epitope in antigenic site B. (From Lambkin, R. and Dimmock, N. J. 1995. *Journal of General Virology* **76**, 889–897.)

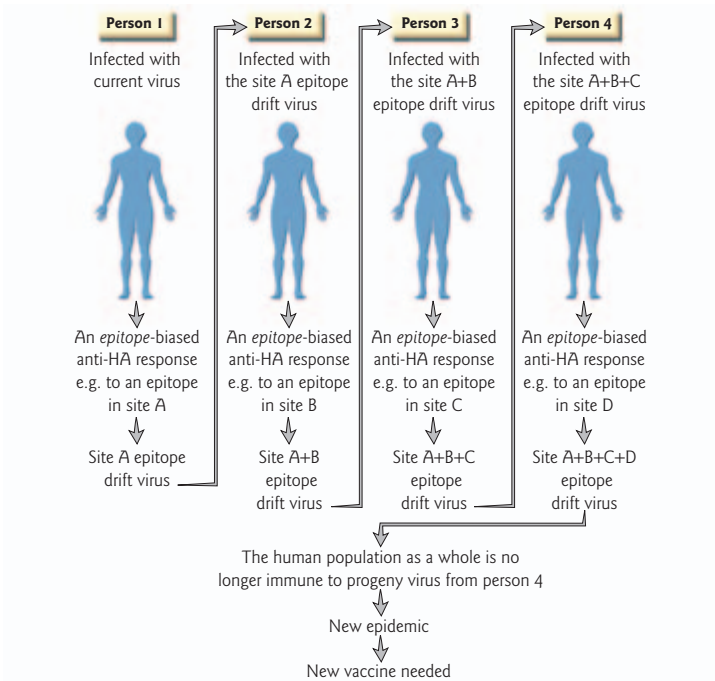

Fig. 17.10 How antigenic drift may occur in nature, bearing in mind that clinically significant drift viruses (that cause epidemics) have four or more amino acid residues changed in two or more antigenic sites present on the hemagglutinin protein. Note that people with biased antibody responses may be uncommon and that there may be any number of nonselective infections of other individuals occurring between the four persons shown here, as indicated by the broken arrow of transmission. A similar process could cause drift of the NA protein.

KEY POINTS

- Viruses are evolutionarily dynamic and respond to changes in the macroenvironment and microenvironment (the body and immune system).
- Every living organism, plant or animal, amoeba or elephant, has its own extensive range of viruses, and new infections of man can arise by nonhuman viruses crossing species, e.g. HIV, influenza A virus. Cross-over is accelerated by loss of habitat and increased proximity of man and animals.
- All established human viruses undergo Darwinian evolution as a result of evolutionary pressure acting on random variants arising by mutation, although the scale of change ranges from, say, measles virus (low) to influenza A virus (high).
- Many new infections in a host species result in high morbidity and mortality, but evolution selects hosts that are genetically more resistant to infection and less virulent progeny virus (e.g. myxoma virus in the European rabbit).
- A new shift variant of influenza A virus could arise at any time and because of its efficient transmission such virus is a major threat to human health. Better antiviral measures than are currently available are needed to combat a virus with the potential of the 1918 virus.

QUESTIONS

- Describe the processes of antigenic drift and antigenic shift in influenza virus and indicate, with reasons, which is likely to generate a more pathogenic virus.
- Using examples, discuss the proposition that viruses with RNA genomes evolve more rapidly than those with DNA genomes.

FURTHER READING

Black, F. L., 1966. Measles endemicity in insular populations: critical community size and its evolutionary implications. *Journal of Theoretical Biology* **11**, 207–211.

Domingo, E., Holland, J. J., 1997. RNA virus mutations and fitness for survival. *Annual Review of Microbiology* **51**, 151–178.

Domingo, E., Webster, R. G., Holland, J. J., 1999. *Origin and Evolution of Viruses*. Academic Press, New York.

Fenner, F., Ratcliffe, F. N., 1965. *Myxomatosis*. Cambridge University Press, London.

Griffiths, P. D., 2004. Should vaccines and antiviral therapy for influenza now be deployed strategically? *Reviews in Medical Virology* **14**, 137–139.

Guan, Y., Poon, L. L. M., Cheung, C. Y. *et al.* 2004. H5N1 influenza: a protean threat. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 8156–8161.

Kobasa, D., Takada, A. E. A., Shinya, K. *et al.* 2004. Enhanced virulence of influenza A viruses with the haemagglutinin of the 1918 pandemic virus. *Nature (London)* **431**, 703–707.

Lamb, R. A., Krug, R. M. 1996. Orthomyxoviridae: the viruses and their replication. In *Fields Virology* (B. N. Fields, D. M. Knipe, and P. M. Howley, eds), Vol. 1, 3rd edn, pp. 1353–1395. Lippincott-Raven, Philadelphia.

Lipatov, A. S., Govorkova, E. A., Webby, R. J. *et al.* 2004. Influenza: emergence and control. *Journal of Virology* **78**, 8951–8959.

Nicholson, K. G., Wood, J. N., Zambon, M. 2003. Influenza. *Lancet* **362**, 1733–1745.

Oxford, J. S. 2000. Influenza A pandemics of the 20th century with special reference to 1918: virology, pathology and epidemiology. *Reviews in Medical Virology* **10**, 119–133.

Reid, A. H., Taubenberger, J. K., Fanning, T. G., 2004. Evidence of an absence: the genetic origins of the 1918 pandemic influenza virus. *Nature Reviews Microbiology* **2**, 909–914.

Webby, R. J., Webster, R. G., 2003. Are we ready for pandemic influenza? *Science* **302**, 1519–1522.

Also check Appendix 7 for references specific to each family of viruses.