

Pathogen evolution within host individuals as a primary cause of senescence

Graham Bell

Biology Department, McGill University, 1205 Ave Dr. Penfield, Montreal, Quebec, Canada H3A 1B1

Received and accepted 22 June 1993

Key words: virus, selection, aging, senescence, HIV, AIDS

Abstract

This paper discusses a novel theory of senescence: the community of pathogens within each host individual evolves during the life-time of the host, and in doing so progressively reduces host vigour. I marshal evidence that asymptomatic host individuals maintain persistent populations of viral pathogens; that these pathogens replicate; that they are often extremely variable; that selection within hosts causes the evolution of pathogens better able to exploit the host; that selection is host-specific; and that such evolving infections cause appreciable and progressive deterioration. Experimental approaches to testing the theory are discussed.

The senescence of clones

The inescapable aging of multicellular organisms might arise in either of two fundamentally different ways. First, the internal capacity to resist and repair damage might be less in older individuals. Because the force of selection declines with age, germ-line mutations which impair function late in life will accumulate more rapidly than those with comparable effects early in life, and mutations which increase vigour early in life may spread even if they have severely deleterious effects later in life. This is the conventional evolutionary explanation of aging, which has received thorough theoretical exploration and substantial experimental support, and which is reviewed at length by other authors in this issue. The second possibility is that the external environment may systematically deteriorate during the lifetime of every individual, so that individuals will senesce even if they are able to maintain a constant level of defence. This paper is concerned exclusively with this possibility, which does not seem to have been investigated previously.

It is obviously out of the question that the physical environment should continually deteriorate during each individual's lifetime, since if this were true

the earth would no longer be capable of supporting life. This is not necessarily true of the biotic environment. Antagonists such as pathogens and their hosts are involved in a continuous process of coevolution, in which the evolution of resistance by the host population is eventually overcome by the evolution of new infective abilities by the pathogen populations, which in turn creates selection for new sources of host resistance, and so on, endlessly. On different time-scales, viruses and bacteria, fungi and helminths will all play this continual game of tag with their hosts. Each new advance made by the pathogen population is conditional: it impairs the vigour of some host genotypes, but not of all, and the host population persists because of the increased proliferation of host genotypes which are not susceptible to the novel pathogen adaptation. Consequently, it is conceivable that the average individual host, at any time, may perceive the environment as continually deteriorating, because of genetic changes in pathogen populations which increase its susceptibility to disease, while the host population is nevertheless able to maintain itself through geological time.

To clarify this idea, consider a thought experiment which contrasts the fate of individuals with

the fate of clones each consisting of many genetically identical individuals. Suppose that we constructed many replicate clones, all of the same genotype, and measured the life table of these replicates, each of which might comprise many individuals, in the same way that we might measure the life table of individuals within any of these clones. If we did measure the life table of individuals belonging to a given clone, we would almost certainly find that the rate of survival (or, in principle, some other component of fitness) decreased with age, indicating that individuals senesce. There is, however, no obvious reason to suspect that this would apply to replicate clones able to propagate themselves through time. It is conceivable that reproduction earlier in time is more important than later reproduction, perhaps because an early-reproducing clone would occupy available space or opportunities more quickly. But it is difficult to see how the reproductive activity of the founders of the clone could systematically reduce the vigour of their descendants, a quite separate set of individuals, many generations later. It seems reasonable to conclude that clones will not senesce, even though each individual member of the clone does. On this basis, we should expect to find that each clone has a fixed probability of becoming extinct in any interval of time, so that the number of surviving clones decreases exponentially through time. Clones with different genotypes might have different rates of extinction, of course; the important point is that for any given genotype this rate would be a constant that did not increase through time. This conclusion will fail if one of two additional processes is operating: if either the genotype itself, or the environment in which it is expressed, tends continually to deteriorate through time.

A clone descending from a single founder will not remain genetically uniform, even if its reproduction is strictly mitotic. Mutation will drive an increase in genetic diversity, until the input of new genetic diversity through mutation is just balanced by the erosion of diversity by selection, at which point the clonally propagated population is in equilibrium, and will maintain the same level of diversity from generation to generation. This argument is not quite true for finite populations, in which the occasional stochastic loss of the class of individuals with the fewest mutations will drive an irreversible increase in the mean number of mutations per indi-

vidual. Because most mutations are somewhat deleterious, the vigour of a finite clone will tend to decrease through time (Muller, 1964; Haigh, 1978). The process can actually be observed in very small clones of asexual protists (Bell, 1988). This paper is not concerned with genetic deterioration of this sort.

A set of replicate clones growing in a natural environment represents a large stationary target for resident populations of parasites and pathogens, which will adapt so as to be able to exploit it more effectively. I assume for simplicity that each clone is completely uniform, removing this restriction later. At first, each clone will survive well, because there has not yet been sufficient time for local pathogens to evolve efficient methods of infection. As time goes on, selection will favour pathogen variants able to exploit this new resource effectively, and as the frequency of such variants increases the survival rate of *all* the replicate clones will fall. Evolution of the local pathogen community will therefore cause a decrease in clonal survival through time; the set of clones will senesce.

It is not known whether such a process of clonal aging occurs in natural populations. There are three possible sources of information. The first is the life table of clones, for organisms in which reproduction produces distinct individuals rather than physically connected ramets or zooids. Claims for the extreme longevity of some clones in plants which reproduce vegetatively, such as creosote bush (Vasek, 1980) and bracken (Olinonen, 1967), are commonplace, but beside the point: large organisms are usually long-lived, but the crucial information on the rate of mortality in relation to age is lacking. Secondly, deliberate experiments in which the survival rate of a clone which is continually replanted into a natural community is compared with that of the same clone maintained in the greenhouse would provide important information (Bell, 1992), but so far as I know none have ever been reported. Finally, accidental experiments are done every time agronomists develop a new clone or inbred line of crop plant and release it for large-scale cultivation. The result is very often a dramatic process of senescence in which the failure of the new variety is quickly brought about by the evolution of local pathogen populations (Barrett, 1981; Wolfe, 1985).

The theory of within-host selection

The relevance of clonal senescence to individual senescence is that multicellular individuals are mitotic clones of cells. The cells may vary dramatically in phenotype, but all are genetically identical, or nearly so. When a pathogen infects a host individual, it may proliferate so successfully that the host is killed quickly by the disease it causes. More often, the growth of the pathogen population is suppressed by host defences, and disease symptoms cease, or never appear. The absence of symptoms is normally taken to imply elimination of the pathogen. Suppose, however, that a small population of the pathogen, made up of somewhat more resistant forms, persists in host tissues. This population continues to reproduce, but is prevented from increasing in numbers, and thereby causing overt symptoms, by host defences. As time goes on, however, variants which are able to replicate somewhat more successfully will be selected in the pathogen population resident in the host. These variants are those pathogen genotypes which happen to be best able to exploit the genotype of the particular host in which they are evolving, and may differ from individual to individual. However, although the pathogen population within the host can evolve adaptation to the host genotype, the host itself cannot evolve, being an individual and not a population. As the pathogen population continues to evolve more effective ways of circumventing host defences, the rate of replication and the abundance of the pathogen will tend to increase, causing a progressive increase in the level of damage sustained by the host, which is macroscopically apparent as a senescent decline in survival or fecundity. Eventually, the host dies, either because the pathogen finally eludes host defences entirely and breaks out to cause acute disease, or because the indirect effects of increased levels of damage caused by evolving pathogens result in the host starving to death or being eaten by a predator.

There is one major complication in this otherwise simple theory that should be addressed before proceeding. Hosts which possess an immune system can evolve, in a sense, to a changing population of pathogens, by producing lymphocytes with altered specificities. The way in which they do this is strikingly similar to the way in which sexual lineages can maintain resistance to external popula-

tions of pathogens - the creation of variation by genetic recombination, followed by the increase in frequency under selection of variants which happen by chance to be resistant. Nevertheless, the range of pathogen genotypes to which a given host genotype can respond effectively is limited, and selection among pathogens can eventually cause the emergence of types able to evade immune surveillance. Immune systems are likely to retard but not to prevent the evolution of increasingly virulent resident populations of pathogens.

The theory that senescence is generally caused by the within-host selection of persistent pathogens requires that at least seven conditions be fulfilled before it can be taken seriously as a rival to the antagonistic-pleiotropy or mutation-accumulation theories.

- (1) It must be demonstrable that all individuals maintain persistent populations of pathogens; ideally, most hosts should be infected with any given common pathogen.
- (2) The pathogen must be capable of horizontal as well as (or rather than) vertical transmission. Parasites which are exclusively vertically transmitted, through the germ line, will be selected for benignity, and will evolve into mutualists such as plastids. Moreover, the pathogen should be horizontally transmitted among cells within the body, rather than being transmitted only by the reproduction of infected cells. This is not an absolute requirement, but greatly enlarges the possibilities of continuing evolution.
- (3) The pathogen must replicate, otherwise selection cannot long continue.
- (4) The pathogen must be variable, to sustain continued selection.
- (5) It must be shown that selection actually occurs, and that it results in populations which are better adapted to exploit the host.
- (6) Selection must be host-specific, since otherwise selection in one host would produce adaptation to many other hosts, and most infections would be well-adapted without the necessity for within-host evolution. Host-specific selection will lead to genetic differences among the pathogen populations in different hosts, even if these hosts were initially infected from the same source.
- (7) Persistent infections must cause appreciable and progressive deterioration.

This onerous list of conditions excludes most pathogens immediately as candidates for producing senescent deterioration. Most metazoan parasites do not replicate within the host. The endogenous bacterial and fungal populations of large perennial plants, and the gut community of animals, are more relevant, but the most promising candidates are mycoplasmas, viruses and retroviruses. I shall now summarize the evidence that viruses and retroviruses, at least, satisfy all of the conditions outlined above. This is in no sense a review of all the relevant literature, which would be virtually a review of the literature of disease. All that I have done is to collate some recent papers, from which the earlier literature can be obtained.

The evolution of viruses within hosts

Viruses can persist in cell cultures for long periods of time. Human parainfluenza virus persisted through 147 passages over 29 months in culture (Murphy, Dimock & Kang, 1990). Polio virus maintained high titres in neuroblastoma cell lines for nine months (Colbere-Garapin *et al.*, 1989). Reovirus readily becomes persistent in epithelial cell lines, causing morphological and physiological changes in the process (Montgomery *et al.*, 1991). Bovine coronavirus continued to replicate in cell culture over a 120-day period of observation (Hofmann, Sethna & Brian, 1990).

Healthy asymptomatic adults are often cryptically infected by many viruses and other pathogens. Jarret *et al.* (1990) found that 18/20 saliva samples from asymptomatic adults contained herpes virus 6; the occasional cell from peripheral blood samples was also infected. A search using polymerase chain reaction of 23 individuals not known to have recently had chicken pox or shingles found that herpes virus was present in nervous tissue from a majority of them (Mahalingam *et al.*, 1990). A survey of over 1000 asymptomatic adults showed that nearly all carried persistent populations of Epstein-Barr virus (Imai, 1990; see also Niedobitek *et al.*, 1991). Maedi and visna viruses often become persistent in sheep (Staskus *et al.*, 1991). Woodchuck often carry persistent infections of a hepatitis virus, and persistent infections can be established by experimental inoculation of intact

virus (Miller *et al.*, 1990). Avian leukosis virus is a transformation-defective variant of Rous sarcoma virus which becomes persistent in ducks despite the continued expression of neutralizing antibodies (Nehyba *et al.*, 1990). Strawberry cultivars thought to be free of virus turned out to carry persistent populations of pallidosis virus (Yoshikawa & Converse, 1990). Prokaryotes can also establish persistent infections with no clinical symptoms. Most mycoplasma infections appear to be symptomless, and may be almost universal. Faden and Dryja (1989) isolated an unusual slow-growing bacterium that would not be detected by conventional tests from human middle-ear fluid. Sunaina, Kishore and Shekhawat (1989) found that healthy potato tubers bore latent populations of *Pseudomonas*, perhaps maintained as symptomless infections of wild plants.

Persistent viruses can replicate. Niedobitek *et al.* (1991) showed that the persistence of Epstein-Barr virus requires continued viral replication, rather than the maturation of previously infected cells. Righthand (1991) showed that persistent non-lytic variants of echovirus 6 replicate. Lemon *et al.* (1991) measured the rate of replication of hepatitis A virus in cultured cells.

Persistent infections can cause continued and progressive deterioration. Persistent infection of the central nervous system by measles virus causes chronic and usually fatal disease (Carrigan & Knox, 1990). Theiler's virus, persistent in the central nervous system of mice, causes a gradual and progressive demyelination (Tangy *et al.*, 1991; Lipton *et al.*, 1991). Persistent viruses of the nervous system have been reviewed by Kristensson and Norrby (1986). Avian leukosis virus and the various hepatitis viruses cause liver necrosis and neoplastic diseases (Nehyba *et al.*, 1990). Continued damage is normally caused only by replicating virus; the amount of damage is proportional to the rate of replication, and persistent viruses, usually with low rates of replication, cause a low level of damage that cumulates through time. Persistent infections by hepatitis B virus are likely to lead to liver disease if the virus is replicating (Fattovich *et al.*, 1990). Coxsackie virus B3 persists in myocardium; the development of myocardial lesions is correlated with the rate of virus replication (Klingel

et al., 1992). Some endogenous retroviruses of mice are initially benign, as expected, but recombinant virus appearing during the lifetime of the host causes thymic lymphoma (Stoye & Coffin, 1985).

Persistent, rather than acute, infection can be caused by simple changes in intact viral genomes. Persistent populations of Theiler's virus in mice are characterized by mutational change in a capsid protein (McAllister *et al.*, 1990; Tangy *et al.*, 1991); a single nucleotide change causing a single amino-acid substitution may direct a shift from acute to persistent infection (Zurbriggen *et al.*, 1991). Similarly, a single amino-acid substitution in the glycoprotein encoded by choriomeningitis virus shifts its behaviour from acute infection followed by clearance to long-term persistence with immune suppression (Matloubian *et al.*, 1990; Salvato *et al.*, 1991). Persistent hepatitis B virus differed from the form present in the initial acute infection by two mutations, one of which prevented antigen expression (Raimondo *et al.*, 1990a).

The altered viruses of persistent infections escape immune surveillance through reduced transcription or reduced replication. Lytic gene expression is reduced or abolished early in the establishment of persistent infections by herpes simplex virus (Kosz-Vnechak, Coen & Knipe, 1990). The persistent forms of pseudorabies virus in swine have lower levels of transcription than those present in acute infections (Lokensgard, Thawley & Molitor, 1990). Persistent strains of Theiler's virus are less abundant and have lower rates of replication than virulent strains (Lipton *et al.*, 1991). Variant forms of hepatitis A virus which appear during persistent infections and which have altered antigenicity, including neutralization resistance to antibody, have lower rates of replication than forms with normal antigenic characteristics (Lemon *et al.*, 1991). A causal relationship between persistence and reduced replication was established by J. S. Li *et al.* (1991), who showed that a mutant of duck hepatitis B virus with a reduced rate of replication established a persistent infection when inoculated experimentally.

Persistence often follows an acute infection. Hepatitis B virus persists in the liver after serological recovery from an acute infection, and is capable

of subsequent reactivation (Ling, Blum & Wands, 1990; see Raimondo *et al.*, 1990b). Mouse hepatitis virus persists in the spinal cord following acute infection, and may subsequently be amplified and spread to cause clinical disease (Perlman *et al.*, 1990). The infection of cultured cells by Theiler's virus kills most of the cells; the virus population in the surviving cells becomes persistent after repeated passage (Patrick *et al.*, 1990).

Virus populations of persistent infections are genetically variable. This has been repeatedly observed since the development of techniques for sequencing nucleic acids; recent examples include parainfluenza virus 3 (Murphy, Dimock & Kang, 1991), hepatitis B virus (Groetzinger & Will, 1992), equine infectious anaemia virus (Salinovich *et al.*, 1986; Alexandersen & Carpenter, 1991), visna virus of sheep (Clements *et al.*, 1980) and caprine arthritis encephalitis virus (Ellis *et al.*, 1987). RNA viruses as a whole are so variable, in large part because the infidelity of RNA polymerases yields a high mutation rate, that their populations should not be thought of as comprising a basic type around which some variation occurs, but rather as a mixture of indefinite and constantly changing composition (Domingo *et al.*, 1985). The literature on retroviral diversity, which may likewise be generated by imprecise replication, has been reviewed by Katz and Skalka (1990).

Viral genomes of persistent infections vary in their ability to attack different cell types. Murine lymphocytic choriomeningitis virus has two sites of infection: the central nervous system, where populations similar to the initial infection cause acute disease, and lymphoid tissue, where altered viruses cause a persistent infection. This difference is associated with a single amino-acid substitution in the viral glycoprotein (Ahmed *et al.*, 1991). Macrophage isolates of this virus tended to grow well in cultured macrophages but poorly in lymphocytes, and vice versa (King *et al.*, 1990), so the occurrence of tissue-specific selection can be modelled in culture. A single passage through the host causes the appearance of tissue-specific variants of bovine herpes virus 1 (Whetstone *et al.*, 1989). The divergence of viral populations in different tissues provides evidence for the occurrence of adaptive evolution within the host.

Some variants in heterogeneous viral populations can escape host defences and are cytopathic. Some variants of measles virus in persistent infections are resistant to interferon (Carrigan & Knox, 1990). Variants of hepatitis A virus isolated from persistently-infected monkey cells were cytopathic (Lemon *et al.*, 1991). The appearance of variants which evade immune surveillance has been reported for visna virus of sheep (Stanley *et al.*, 1988) and for equine infectious anaemia virus (Salinovich *et al.*, 1986).

Selection can cause rapid and substantial change in the genetic composition of viral populations within cell cultures or hosts. Genetic changes can be detected in bovine herpes virus 1 after a single passage through the host (Whetstone *et al.*, 1989). Such changes cumulate through time. Diez *et al.* (1990) report the structure of part of the genome of foot-and-mouth disease virus after 100 passages in cell culture. Only 5/15 mutations were silent. There were nine amino-acid substitutions in the capsid protein, including some at sites which in other picornaviruses are highly conserved; thus, even highly-conserved sites can evolve when viral populations are exposed to selection in a novel environment. The evidence for selection in retroviral populations has been reviewed by Katz and Skalka (1990).

Selection may favour variants with reduced virulence and cytotoxicity in cell culture. This is often associated with the appearance of incomplete viral genomes in the form of so-called defective interfering particles, for example in human parainfluenza virus (Moscona, 1991), Murray Valley encephalitis virus (Poidinger, Coelen & MacKenzie, 1991), and tomato bushy stunt virus (Knorr *et al.*, 1991). Since these are parasites of intact viruses, their effect on the host is difficult to predict. However, in many cases selection alters the population of intact virus. Rubella virus maintained with antibody in cell culture evolved reduced replication and cytotoxicity; these changes took place in intact virus, though defective interfering RNAs also appeared (Abernathy, Wang & Frey, 1990). The passage of lymphocytic choriomeningitis virus through cultured mouse cells results in the evolution of non-pathogenic populations with altered genomes (Bruns *et al.*, 1990). Pelletier *et al.* (1991) selected persistent

mutants of polio virus in cultured cells, obtaining forms that could persist in non-neural tissue and had altered cell specificity. Measles virus populations may contain two forms resistant to interferon, one of which replicates normally and destroys cells, while the other has reduced replication and cytopathogenicity. The passage of populations containing both forms through nervous tissue of hamsters selects strongly for the less damaging form, causing chronic persistence of the virus. The outcome of such processes depends on the balance between vertical transmission (through the reproduction of infected cells) and horizontal transmission (through the movement of virus from infected to uninfected cells). Reducing the importance of horizontal transmission, for example by the presence of soluble antibody, will shift the balance towards vertical transmission and favour the evolution of benignity. The study of hepatitis A virus by Lemon *et al.* (1991) is a particularly clear description of the mechanism involved: altered forms with reduced antigenicity have greater rates of replication during persistent infection, while normal forms have greater rates during serial passage.

Selection also occurs in the living host. Groetzinger and Will (1992) comment that: 'There is increasing evidence for selection of hepatitis B virus mutants during the natural course of infection, after immunization, and during immune therapy. These mutants can prolong viral persistence, escape immune-mediated elimination, and influence the clinical sequelae of infection.' The appearance of genetically distinct persistent forms in living hosts is, of course, strong evidence for selection.

The outcome of selection may depend on host genotype and on cell type. The ability of herpes virus to establish persistent populations in mammalian hosts varies with the species and strain of the host (Gordon *et al.*, 1990). Borneo disease virus causes encephalitis in most strains of rat, but in one strain no clinical disease is apparent, despite persistent replication of the virus in the central nervous system of the host (Herzog, Frese & Rott, 1991). The mutagenesis of mouse fibroblasts readily produced cell lines resistant to mouse hepatitis virus, through reduced susceptibility to membrane fusion encoded by a viral glycoprotein (Daya *et al.*, 1990). It is probable, therefore, that the final genetic composi-

tion of viral populations, after selection, will vary among host individuals, each host presenting a different genetic environment for viral evolution. Kato *et al.* (1992) found extensive variation among hepatitis C virus isolated from different patients with respect to envelope protein genes.

These results suggest that a wide range of viruses, and perhaps other pathogens, develop highly variable populations within their hosts, and in consequence undergo selection for persistent types which through continued evolution produce cumulative and eventually substantial damage. The most detailed and extensive studies of variation and within-host selection, however, have concerned the human immunodeficiency virus HIV and its relatives, slow viruses which may persist asymptotically for many years after an initial acute phase.

The special case of immunodeficiency viruses

Populations of HIV within single host individuals are extremely heterogeneous. (Hahn *et al.*, 1986; Saag *et al.*, 1988). There is extensive sequence variation in the *env* gene, which encodes envelope glycoproteins (see Howell *et al.*, 1991), with frequent amino-acid substitutions at sites critical in antibody and T-cell recognition (Simmonds *et al.*, 1990; Callahan *et al.*, 1990). Hypervariable sites in *env* of HIV-1 correspond to open regions of RNA secondary structure with little intramolecular base-pairing, which are mostly extracellular portions of the molecule (Le *et al.*, 1989). The substitution of a single amino-acid in such regions can reduce antibody binding specificity (Looney *et al.*, 1989; McKeating *et al.*, 1989). Sites which direct antagonistic interactions with the host are thus particularly variable and liable to change readily under selection (see Simmonds *et al.*, 1990; Balfe *et al.*, 1990).

High levels of variation have also been reported for *nef* (Delassus, Chenier & Wain-Hobson, 1991) and *rev* (Martins *et al.*, 1991) in cell culture, and for *env* of the related simian immunodeficiency virus (Burns & Desrosiers, 1991). The very general operation of selection in viral populations itself casts into doubt the relevance of culture studies, in which populations are exposed to a novel environment. However, the extreme heterogeneity of HIV has been confirmed *in vivo*. Y. Li *et al.* (1991) characterized ten genomes obtained directly from uncul-

tured brain tissue of an AIDS patient and found four full-length genomes with one or two LTRs, three defective genomes with internal deletions, two rearranged genomes with inverted LTRs, and an inverted proviral half flanked by host sequences. Transfection of the four full-length genomes showed that one was fully able to replicate and was comparable with cultured virus. Thus, HIV populations within the host include both fully replicating forms, which themselves show sequence variation, and complex mixtures of other viral entities.

HIV populations in different tissues are genetically different. The frequency of different variant sequence differs among brain, spleen, lymph and blood isolates (Epstein *et al.*, 1991; Haggerty & Stevenson, 1991), which differ from the populations found in cultured cells (Pang *et al.*, 1991). Terwilliger *et al.* (1991) found that allelic variation in *nef* differentially affected the ability of HIV-1 to replicate in different cell lines.

Selection occurs in HIV populations during serial passage in cell culture. Types which are initially rare may increase greatly in frequency (Zweig *et al.*, 1990; Vartanian *et al.*, 1991). Specific variants resistant to drugs are readily selected (Gao *et al.*, 1992). More importantly, passage in the presence of soluble antibody causes the evolution of resistant populations (Robert-Guroff *et al.*, 1986; Masuda *et al.*, 1990; McKeating *et al.*, 1991; Ashkenazi *et al.*, 1991; Rey *et al.*, 1991), and may occur even in highly conserved regions of the viral genome. Hoxie *et al.* (1991) found that the passage of attenuated HIV-1 with low binding affinity for CDA cells eventually resulted in the appearance of cytopathic variants whose binding affinities were comparable with those of normal cytopathic HIV-2 isolates. It is likely that there are several different routes by which resistance can be acquired, since it may or may not be associated with decreased binding affinity to soluble antibody (compare McKeating *et al.*, 1991, with Ashkenazi *et al.*, 1991). L'Age-Stehr *et al.* (1990) observed the evolution of a shorter latent period and increased virulence towards T-cells when HIV was maintained in cell culture.

Damage caused to the host is related to levels of viral replication. Rapidly-replicating isolates which induce syncytium formation characterize in-

dividuals who progress rapidly to AIDS and tend to die soon; individuals who remain asymptomatic or survive longer yield more slowly-replicating isolates (Tersmette *et al.*, 1990). The replication rate of HIV is correlated with the rate of loss of CDA+ lymphocytes (Tersmette *et al.*, 1989).

Sequence variation among tissues seems to be caused by tissue-specific selection. Peden, Emerman and Montagnier (1991) cultured isolates from three individuals on six different cell lines and found that replicative ability and tropism changed after repeated passage, causing divergence from a single ancestral sequence. The variety of tissues that can be infected by HIV increases during the lifetime of the host (Cheng-Mayer *et al.*, 1988).

Different loci can evolve independently as the result of recombination. Masuda and Harada (1990) obtained recombinant virus after co-culturing two distinct strains of HIV, and recombination seems to be a major source of heterogeneity of HIV populations within single hosts (Vartanian *et al.*, 1991; Delassus, Chenier & Wain-Hobson, 1991; Howell *et al.*, 1991). The three loci scored over four years from the same patient by Martins *et al.* (1991) all behaved differently.

Longitudinal studies provide convincing evidence for selection and evolution within single host individuals. Phillips *et al.* (1991) found that fluctuations in the specificity of cytotoxic T-cells for virus were matched by variability in proviral *gag* DNA epitope sequences. Some of these variants were not recognised by T-cells, and the accumulation of such variants would provide a basis for immune escape. Wolfs *et al.* (1991) studied *env* sequences from two individuals over five years. At the time of antibody seroconversion, genomic RNA levels peaked and the virus population was highly homogeneous. During the course of infection, heterogeneity increased and the consensus sequence shifted. A single amino-acid substitution was fixed in both patients; this substitution affected antibody binding specificity. After a subsequent increase of genomic RNA and progression to AIDS in one patient, a new variant with major changes in the principal neutralization domain of the viral genome emerged, again forming a homogeneous population.

Host-specific selection leads to variation among host individuals. The most detailed studies concern the *env* gene, and especially the hypervariable V3 domain of the envelope glycoprotein gp120 which it encodes. Extensive variation among individuals has been documented by Simmonds *et al.* (1990) and Epstein *et al.* (1991). The virus populations of six children infected from a single source diverged rapidly from the initial inoculum in different directions (Wolfs *et al.*, 1990).

Within-host selection in relation to other theories

The material that I have reviewed above shows that all the preconditions required by the hypothesis of within-host selection are met, at least in some instances. Whether or not they are *generally* true must await the accumulation of evidence. However, the availability of methods for amplifying viral genomes present at very low abundance in host tissues will make it possible to decide very soon whether the single most important and most surprising observation which the theory requires, the universality of persistent viral infection, is really the case.

Should all the conditions be shown to hold generally, it will still remain to be shown that they operate so as to produce senescence. An obvious experimental approach is to rear rigorously pathogen-free stocks by isolating sterile eggs and raising them in enclosed or filtered environments. The suggestion that senescence is caused by the diseases associated with internal populations of microbes was first made by Metchnikoff in the early 1900s. He emphasized the deleterious nature of toxins produced by the bacterial population of the large bowel, but also described the pathological behaviour of lymphocytes (whose phagocytic properties he discovered) and thus autoimmune diseases. His theory was quite different from that being advanced here: he attributed senescence to the long-continued action of bacterial toxins without reference to the possibility of pathogen evolution within the host. It was thought to be adequately refuted by the observation that animals reared in bacteria-free conditions were not immortal, and indeed usually died without notably exceeding their normal span. The current neglect of his ideas may not be completely justified. For example, Greenberg and

Burkman (1963) compared bacteria-free with normal adult flies, recording average lifespan for both sexes in two experiments. There was no difference between the two groups in two comparisons, but in the remaining two (concerning females in one case and males in the other) the bacteria-free flies lived much longer. However, I have been unable to find observations of the longevity of defined strains of animal under bacteria-free conditions which would support the crucial test of whether rates of mortality increase with age when cellular pathogens are excluded. A detailed quantitative life table for a colony of bacteria-free mice would be of great interest.

In this paper I have emphasized the role of viruses rather than bacteria in forming persistent populations within the host. It is possible, though very difficult, to exclude exogenous viruses, and maintain stocks of animals in completely axenic conditions. Some virus will be transmitted in the egg, of course, but because elements which are exclusively vertically transmitted are not expected to be pathogenic (mouse mammary tumour virus seems to be an exception), this is not necessarily a problem in principle. If such stocks could be shown to be virus-free and did not senesce, the theory would be supported. In practice, the possibility that some retroviruses are partly horizontally and partly vertically transmitted, and that purely endogenous viruses might in time evolve exogenous variants, might cloud the interpretation of a negative result. I have been unable to find any information on the lifespan of animals reared in virus-free conditions.

A more ingenious and more practicable experiment might test the prediction that within-host selection causes the evolution of more pathogenic strains, using genetically uniform host individuals produced asexually. Inoculation of a young host with viral isolates from an old member of the same clone should cause more rapid senescence. Moreover, this should not be the case when the inoculum is obtained from an unrelated individual. I do not know of any experiments of this kind.

It has been reported that individuals do not senesce appreciably in worms which proliferate vegetatively by a nearly equal binary fission (Bell, 1985). This has been taken to support the antagonistic-pleiotropy or mutation-accumulation hypotheses, because it will not be possible to select for the postponement of deleterious gene expression when parent and offspring are indistinguishable and

therefore of the same age. At the same time, it seems to negate the hypothesis of within-host selection, which will occur in any piece of tissue, regardless of its status as parent or offspring. It seems desirable to repeat this observation, which has been criticized by Martinez and Levinton (1992), who report the occurrence of senescence in an annelid reproducing by fission. If it turns out to be generally true, it offers strong support for the conventional view and strong evidence against the contrary view being advanced in this paper.

The hypothesis of within-host selection, on the one hand, and related hypotheses of antagonistic pleiotropy and mutation accumulation, on the other hand, are quite different, but they are not mutually exclusive. Rather, they might complement one another in either of two ways. First, the reduced levels of maintenance and repair in older individuals that evolve as the consequence of the decrease in the force of natural selection with increasing age will make it easier for persistent populations of pathogens to evolve resistance to host defences. Secondly, the prospect that persistent pathogen populations will become steadily better adapted during the lifetime of the host further devalues the maintenance of vigour in old individuals, and therefore advances the schedules of antagonistic pleiotropy and mutation accumulation.

A very similar theory has been developed for the particular case of HIV infection by Nowak (1992; Nowak & May, 1991). This virus represents a special case because it attacks the very cells that are responsible for its suppression, and in consequence the dynamics of its evolution in the host are more complex. Nowak's theory differs from the simpler theory advanced in this paper in that the heterogeneity of the viral population plays a central role in disease progression, rather than merely providing a basis for selection of more effective variants. The crucial concept is that the immune cells whose production is specifically induced by a given viral genotype are active only against that genotype, whereas they can themselves be killed by any intact virus. It follows that more heterogeneous viral populations will suppress the immune response more effectively, and that there exists a threshold value of viral diversity beyond which the immune system is incapable of restraining the virus and preventing progression to AIDS. It is likely that the immune response is not completely specific, so that new

clones of immune cells are active not only against the particular viral genotype responsible for their induction, but also against a range of other genotypes. If viral genotypes exist which can survive a non-specific response (when this is not true, the initial infection is quickly cleared) but not the combined effect of the specific and non-specific responses, then there will be an initial viremia, perhaps accompanied by clinical symptoms, followed by a reduction in virus titre and a long and variable period of persistence before the viral diversity threshold is exceeded and a final phase of acute disease ensues. Differential-equation models of this process are strikingly successful in capturing the major features of the time-course of HIV infection. Whether they are preferable to the simpler theory of the sequential selection of more effective pathogen genotypes from a population whose diversity is maintained by high mutation rates is not clear. One possible approach might be to construct the phylogeny of the evolving viral population, because simple within-host selection implies the sequential replacement of currently abundant variants by slightly modified descendents, whereas Nowak's theory implies that there is no single successful line of descent. Alternatively, simple within-host selection would lead us to expect that the final HIV population causing AIDS would be more homogeneous than the persistent population during the asymptomatic phase, since the single viral strain able to overcome host defences would spread to fixation; this seems to correspond with the situation described by Wolfs *et al.* (1991). Nowak's theory leads us to expect, to the contrary, that the final HIV population will be maximally heterogeneous. However, he suggests that the breakdown of immune function which is eventually caused by increasing viral diversity itself permits rapidly-replicating viral genotypes to be selected, creating genetic uniformity as a consequence rather than a cause of the development of acute disease. This may well be the case; the coupled dynamics of HIV infection and the immune system may necessitate a more complicated explanation than is required for other diseases. The more general significance of the experimental and theoretical work on HIV is that it provides a dramatic illustration of how evolutionary processes driven by within-host selection cause a cumulative and eventually catastrophic collapse of host vigour.

References

- Abernathy, E. S., C. Y. Wang & T. K. Frey, 1990. Effect of antiviral antibody on maintenance of long-term rubella virus persistent infection in Vero cells. *Journal of Virology* 64: 5183-5187.
- Ahmed, R., C. S. Hahn, T. Somasundaram, L. Villarete, M. Matloubian & J. H. Strauss, 1991. Molecular basis of organ-specific selection of viral variants during chronic infection. *Journal of Virology* 65: 4242-4247.
- Alexandersen, S. & S. Carpenter, 1991. Characterization of variable regions in the envelope and S3 open reading frame of equine infectious anemia virus. *Journal of Virology* 65: 4255-4262.
- Ashkenazi, A., D. H. Smith, S. A. Marsters, L. Riddle, T. J. Gregory, D. D. Ho & D. J. Capon, 1991. Resistance of primary isolates of human immunodeficiency virus type 1 to soluble CD4 is independent of CD4-rgp120 binding affinity. *Proceedings of the National Academy of Sciences of the United States of America* 88: 7056-7060.
- Balfe, P., P. Simmonds, C. A. Ludlam, J. O. Bishop & A. J. Leigh-Brown, 1990. Concurrent evolution of HIV-1 in patients infected from the same source. *Journal of Virology* 64: 6221-6231.
- Barrett, J. A., 1981. The evolutionary consequences of monoculture, pp. 209-248 in *Genetic Consequences of Man-made Change*, edited by J. A. Bishop and L. M. Cook. Academic Press, London.
- Bell, G., 1985. Evolutionary and non-evolutionary theories of senescence. *The American Naturalist* 124: 600-603.
- Bell, G., 1988. *Sex and Death in Protozoa: the History of an Obsession*. Cambridge University Press.
- Bell, G., 1992. Five properties of environments, pp. 33-56 in *Molds, Molecules and Metazoa*, edited by P. R. Grant and H. S. Horn. Princeton University Press.
- Bruns, M., T. Krazberg, W. Zeller & F. Lehmann-Grube, 1990. Mode of replication of lymphocytic choriomeningitis virus in persistently infected cultivated mouse L cells. *Virology* 177: 615-624.
- Burns, D. P. W. & R. C. Desrosiers, 1991. Selection of genetic variants of simian immunodeficiency virus in persistently infected rhesus monkeys. *Journal of Virology* 65: 1843-1854.
- Callahan, K. M., M. M. Fort, E. A. Obah, E. L. Reinherz & R. F. Siliciano, 1990. Genetic variability in HIV-1 gp120 affects interactions with HLA molecules and T cell receptor. *Journal of Immunology* 144: 3341-3346.
- Carrigan, D. R. & K. K. Knox, 1990. Identification of interferon-resistant subpopulations in several strains of measles virus: Positive selection by growth of the virus in brain tissue. *Journal of Virology* 64: 1606-1615.
- Cheng-Mayer, C., D. Seto, M. Tateno & J. A. Levy, 1988. Biological features of HIV-1 that correlate with variation in the host. *Science* 240: 80-82.
- Clements, J. E., F. S. Pedersen, O. Narayan & W. A. Haseltine, 1980. Genomic changes associated with antigenic variation of visna virus during persistent infection. *Proceedings of the National Academy of Sciences of the United States of America* 77: 4454-4458.
- Colbere-Garapin, F., C. Christodoulou, R. Crainic & I. Pelletier,

1989. Persistent poliovirus infection of human neuroblastoma cells. *Proceedings of the National Academy of Sciences of the United States of America* 86: 7590-7594.
- Daya, M., F. Wong, M. Cervin, G. Evans, H. Vennema, W. Spaan & R. Anderson, 1989. Mutation of host cell determinants which discriminate between lytic and persistent mouse hepatitis virus infection results in a fusion-resistant phenotype. *Journal of General Virology* 70: 3335-3346.
- Delassus, S., R. Cheyner & S. Wain-Hobson, 1991. Evolution of human immunodeficiency virus type 1 nef and long terminal repeat sequences over 4 years *in vivo* and *in vitro*. *Journal of Virology* 65: 225-231.
- Diez, J., M. Davila, C. Escarmis, M. G. Mateu, J. Dominguez, J. J. Perez, E. Giralt, J. A. Melero & E. Domingo, 1990. Unique amino acid substitutions in the capsid proteins of foot-and-mouth disease virus from a persistent infection in cell culture. *Journal of Virology* 64: 5519-5528.
- Domingo, E., E. Martinez-Salas, F. Sobrino, J. C. de la Torre, A. Portela *et al.*, 1985. The quasispecies (extremely heterogeneous) nature of viral RNA genome populations: biological relevance - a review. *Gene* 40: 1-8.
- Ellis, T. M., G. E. Wilcox & W. F. Robinson, 1987. Antigenic variation of CAEV during persistent infection of goats. *Journal of General Virology* 68: 3145-3152.
- Epstein, L. G., C. Kuiken, B. M. Blumberg, S. Hartman, L. R. Sharer, M. Clement & J. Goudsmit, 1991. HIV-1 V3 domain variation in brain and spleen of children with AIDS: Tissue-specific evolution within host-determined quasispecies. *Virology* 180: 583-590.
- Faden, H. & D. Dryja, 1989. Recovery of a unique bacterial organism in human middle ear fluid and its possible role in chronic otitis media. *Journal of Clinical Microbiology* 27: 2488-2491.
- Fattovich, G., L. Brollo, A. Alberti, G. Giustina, P. Pontisso, G. Realdi & A. Ruol, 1990. Chronic persistent hepatitis type B can be a progressive disease when associated with sustained virus replication. *Journal of Hepatology (Amsterdam)* 11: 29-33.
- Gao, Q., Z. Gu, M. A. Parniak, X. Li & M. A. Wainberg, 1992. *In vitro* selection of variants of human immunodeficiency virus type 1 resistant to 3'-azido-3'-deoxythymidine and 2',3'-dideoxyinosine. *Journal of Virology* 66: 12-19.
- Gordon, J. J., J. L. C. McKnight, J. M. Ostrove, E. Romanowski & T. Araullo-Cruz, 1990. Host species and strain differences affect the ability of an HSV-1 ICPO deletion mutant to establish latency and spontaneously reactivate *in vivo*. *Virology* 178: 469-477.
- Greenberg, B. & A. M. Burkman, 1963. Effect of B-vitamins and a mixed flora on the longevity of germ-free adult houseflies, *Musca domestica* L. *Journal of cellular and comparative Physiology* 62: 17-22.
- Groetzinger, T. & H. Will, 1992. Sensitive method for identification of minor hepatitis B mutant viruses. *Virology* 187: 383-387.
- Haggerty, S. & M. Stevenson, 1991. Predominance of distinct viral genotypes in brain and lymph node compartments of HIV-infected individuals. *Viral Immunology* 4: 123-132.
- Hahn, B. H., G. M. Shaw, M. E. Taylor, R. R. Redfield, P. D. Markham, S. Z. Salahuddin, F. Wong-Staal, R. C. Gallo, E. S. Parks & W. P. Parks, 1986. Genetic variation in HTLVIII/LAV over time in patients with AIDS or at risk for AIDS. *Science* 232: 1548-1553.
- Haigh, J., 1978. The accumulation of deleterious genes in a population-Muller's Ratchet. *Theoretical Population Biology* 14: 251-267.
- Herzog, S., K. Frese & R. Rott, 1991. Studies on the genetic control of resistance of black hooded rats to Borna disease. *Journal of General Virology* 72: 535-540.
- Hofmann, M. A., P. B. Sethna & D. A. Brian, 1990. Bovine coronavirus messenger RNA replication continues throughout persistent infection in cell culture. *Journal of Virology* 64: 4108-4114.
- Howell, R. M., J. E. Fitzgibbon, M. Noe, Z. Ren, D. J. Gocke, T. A. Schwartz & D. T. Dubin, 1991. *In vivo* sequence variation of the human immunodeficiency virus type 1 *env* gene: Evidence for recombination among variants found in a single individual. *AIDS Research and Human Retroviruses* 7: 869-876.
- Hoxie, J. A., L. F. Brass, C. H. Pletcher, B. S. Haggarty & B. H. Hahn, 1991. Cytopathic variants of an attenuated isolate of human immunodeficiency virus type 2 exhibit increased affinity for CD4. *Journal of Virology* 65: 5096-5101.
- Imai, S., 1990. Virological and immunological studies on inapparent Epstein-Barr virus infection in healthy individuals: In comparison to immunosuppressed patients and patients with infectious mononucleosis. *Hokkaido Journal of Medical Science* 65: 481-492.
- Jarrett, R. F., D. A. Clark, S. F. Josephs & D. E. Onions, 1990. Detection of human herpesvirus-6 DNA in peripheral blood and saliva. *Journal of Medical Virology* 32: 73-76.
- Kato, N., Y. Ootsuyama, T. Tanaka, M. Nakagawa, T. Nakazawa, K. Muraio, S. Ohkoshi, M. Hijikata & K. Shimotohno, 1992. Marked sequence diversity in the putative envelope proteins of hepatitis C viruses. *Virus Research* 22: 107-123.
- Katz, R. A. & A. M. Skalka, 1990. Generation of diversity in retroviruses. *Annual Review of Genetics* 24: 409-445.
- King, C. C., R. De-Fries, S. R. Kolhekar & R. Ahmed, 1990. *In vivo* selection of lymphocyte-tropic and macrophage-tropic variants of lymphocytic choriomeningitis virus during persistent infection. *Journal of Virology* 64: 5611-5616.
- Klingel, K., C. Hohenadl, A. Canu, M. Albrecht, M. Seemann, G. Mall & R. Kandolf, 1992. Ongoing enterovirus-induced myocarditis is associated with persistent heart muscle infection: Quantitative analysis of virus replication, tissue damage, and inflammation. *Proceedings of the National Academy of Sciences of the United States of America* 89: 314-318.
- Knorr, D. A., R. H. Mullin, P. Q. Hearne & T. J. Morris, 1991. De novo generation of defective interfering RNAs of tomato bushy stunt virus by high multiplicity passage. *Virology* 181: 193-202.
- Kosz-Vnechak, M., D. N. Coen & D. M. Knipe, 1990. Restricted expression of herpes simplex virus lytic genes during establishment of latent infection by thymidine kinase-negative mutant viruses. *Journal of Virology* 64: 5396-5402.
- Kristensson, K. & E. Norrby, 1986. Persistence of RNA viruses in the central nervous system. *Annual Reviews of Microbiology* 40: 159-184.
- L'Age-Stehr, J., M. Niedrig, H. R. Gledlerblom, J. W. Sim-

- Brandenburg, M. Urban-Schriefer, E. P. Rieber, J. G. Haas, G. Riethmueller & H. W. L. Ziegler-Heitbrock, 1990. Infection of the human monocytic cell line Mono Mac6 with human immunodeficiency virus types 1 and 2 results in long-term production of virus variants with increased cytopathogenicity for CD4-positive T cells. *Journal of Virology* 64: 3982-3987.
- Le, S. Y., J. H. Chen, D. Chatterjee & J. V. Maizel, 1989. Sequence divergence and open regions of RNA secondary structures in the envelope regions of the 17 human immunodeficiency virus isolates. *Nucleic Acids Research* 17: 3275-3288.
- Lemon, S. M., P. C. Murphy, P. A. Shields, L. H. Ping, S. M. Feinstone, T. Cromeans & R. W. Jansen, 1991. Antigenic and genetic variation in cytopathic hepatitis A virus variants arising during persistent infection: Evidence for genetic recombination. *Journal of Virology* 65: 2056-2065.
- Li, J. S., I. Fouré, C. Jaquet & C. Trepo, 1991. Decreased replication capacity of a duck hepatitis B virus mutant with altered distal pre-S region. *Virus Research* 20: 11-22.
- Li, Y., J. C. Kappes, J. A. Conway, R. W. Price, G. M. Shaw & B. H. Hahn, 1991. Molecular characterization of human immunodeficiency virus type 1 cloned directly from uncultured human brain tissue: Identification of replication-competent and replication-defective viral genomes. *Journal of Virology* 65: 3973-3985.
- Lipton, H. L., M. Calenoff, P. Bandyopadhyay, S. D. Miller, M. C. Dal-Canto, S. Gerety & K. Jensen, 1991. The 5' noncoding sequences from a less virulent Theiler's virus dramatically attenuate GDVII neurovirulence. *Journal of Virology* 65: 4370-4377.
- Lokensgard, J. R., D. G. Thawley & T. W. Molitor, 1990. Pseudorabies virus latency: Restricted transcription. *Archives of Virology* 110: 129-136.
- Looney, D. J., A. G. Fisher & S. D. Putney, 1988. Type-restricted neutralization of molecular clones of HIV. *Science* 241: 357-359.
- Mahalingam, R., M. Wellish, W. Wolf, A. N. Dueland, R. Cohrs, A. Vafai & D. Gilden, 1990. Latent varicella-zoster viral DNA in human trigeminal and thoracic ganglia. *New England Journal of Medicine* 323: 627-631.
- Martinez, D. E. & J. S. Levinton, 1992. Asexual metazoans undergo senescence. *Proceedings of the National Academy of the USA* 89: 9920-9923.
- Martins, L. P., N. Chenciner, B. Asjo, A. Meyerhans & S. Wain-Hobson, 1991. Independent fluctuation of human immunodeficiency virus type 1 rev and gp41 quasispecies in vivo. *Journal of Virology* 65: 4502-4507.
- Masuda, T., S. Matsushita, M. J. Kuroda, M. Kannagi, K. Takatsuki & S. Harada, 1990. Generation of neutralization-resistant HIV-1 *in vitro* due to amino acid interchanges of third hypervariable env region. *Journal of Immunology* 145: 3240-3246.
- Matloubian, M., T. Somasundaram, S. R. Kolhekar, R. Selvakumar & R. Ahmed, 1990. Genetic basis of viral persistence: Single amino acid change in the viral glycoprotein affects ability of lymphocytic choriomeningitis virus to persist in adult mice. *Journal of Experimental Medicine* 172: 1043-1048.
- McAllister, A., F. Tangy, C. Aubert & M. Brahic, 1990. Genetic mapping of the ability of Theiler's virus to persist and demyelinate. *Journal of Virology* 64: 4252-4257.
- McKeating, J., P. Balfe, P. Clapham & R. A. Weiss, 1991. Recombinant CD4-selected human immunodeficiency virus type 1 variants with reduced gp120 affinity for CD4 and increased cell fusion capacity. *Journal of Virology* 65: 4777-4785.
- Metchnikoff, E., 1904. *The Nature of Man*. [I have read the 1938 translation of Sir P. Chalmers Mitchell, published by Watts & Co, London.]
- Miller, R. H., R. Girones, P. J. Cote, W. E. Hornbuckle, T. Chestnut, B. H. Baldwin, B. E. Korba, B. C. Tennant, J. L. Gerin & R. H. Purcell, 1990. Evidence against a requisite role for defective virus in the establishment of persistent hepatitis B virus infections. *Proceedings of the National Academy of Sciences of the United States of America* 87: 9329-9332.
- Montgomery, L. B., C. Y. Y. Kao, E. Verdin, C. Cahill & E. Maratos-Flier, 1991. Infection of a polarized epithelial cell line with wild-type reovirus leads to virus persistence and altered cellular functions. *Journal of General Virology* 72: 2939-2946.
- Moscona, A., 1991. Defective interfering particles of human parainfluenza virus type 3 are associated with persistent infection in cell culture. *Virology* 183: 821-824.
- Muller, H. J., 1964. The relation of recombination to mutational advance. *Mutation Research* 1: 2-9.
- Murphy, D. G., K. Dimock & C. Y. Kang, 1990. Viral RNA and protein synthesis in two LLC-MK-2 cell lines persistently infected with human parainfluenza virus 3. *Virus Research* 16: 1-16.
- Murphy, D. G., K. Dimock & C. Y. Kang, 1991. Numerous transitions in human parainfluenza virus 3 RNA recovered from persistently infected cells. *Virology* 181: 760-763.
- Nehyba, J., J. Svoboda, I. Karakoz, J. Geryk & J. Hejnar, 1990. Ducks: A new experimental host system for studying persistent infection with avian leukemia retroviruses. *Journal of General Virology* 71: 1937-1946.
- Niedobitek, G., L. S. Young, R. Lau, L. Brooks, D. Greenspan, J. S. Greenspan & A. B. Rickinson, 1991. Epstein-Barr virus infection in oral hairy leukoplakia: Virus replication in the absence of a detectable latent phase. *Journal of General Virology* 72: 3035-3046.
- Nowak, M. A., 1992. Variability of HIV infections. *Journal of Theoretical Biology* 155: 1-20.
- Nowak, M. A. & R. M. May, 1991. Mathematical biology of HIV infections: antigenic variation and diversity threshold. *Mathematical Biosciences* 106: 1-21.
- Oinonen, E., 1967. Sporal regeneration of bracken in Finland in the light of the dimension and age of its clones. *Acta Forest Fennica* 83: 1-96.
- Pang, S., H. V. Vinters, T. Akashi, W. A. O'Brien & I. S. Y. Chen, 1991. HIV-1 env sequence variation in brain tissue of patients with AIDS-related neurologic disease. *Journal of Acquired Immune Deficiency Syndromes* 4: 1082-1092.
- Patrick, A. K., E. L. Oleszak, J. L. Leibowitz & M. Rodriguez, 1990. Persistent infection of a glioma cell line generates a Theiler's virus variant which fails to induce demyelinating disease in SJL/J mice. *Journal of General Virology* 71: 2123-2132.

- Peden, K., M. Emerman & L. Montagnier, 1991. Changes in growth properties on passage in tissue culture of viruses derived from infectious molecular clones of HIV-1-LAI, HIV-1-MAL, and HIV-1-ELI. *Virology* 185: 661-672.
- Pelletier, I., T. Couderc, S. Borzakian, E. Wyckoff, R. Crainic, E. Ehrenfeld & F. Colbere-Garapin, 1991. Characterization of persistent poliovirus mutants selected in human neuroblastoma cells. *Virology* 180: 729-737.
- Perlman, S., G. Jacobsen, A. L. Olson & A. Afifi, 1990. Identification of the spinal cord as a major site of persistence during chronic infection with a murine coronavirus. *Virology* 175: 418-426.
- Phillips, R. E., S. Rowland-Jones, D. F. Nixon, F. M. Gotch, J. P. Edwards, A. O. Ogunlesi, J. G. Elvin, J. A. Rothbard & C. R. M. Bangham *et al.*, 1991. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. *Nature* (London) 354: 453-459.
- Poidinger, M., R. J. Coelen & J. S. Mackenzie, 1991. Persistent infection of Vero cells by the flavivirus Murray Valley encephalitis virus. *Journal of General Virology* 72: 573-578.
- Raimondo, G., R. Schneider, M. Stemler, V. Smedile, G. Rodino & H. Will, 1990a. A new hepatitis B virus variant in a chronic carrier with multiple episodes of viral reactivation and acute hepatitis. *Virology* 179: 64-68.
- Raimondo, R., M. Stemler, R. Schneider, G. Wildner, G. Squadraro & H. Will, 1990b. Latency and reactivation of a precore mutant hepatitis B virus in a chronically infected patient. *Journal of Hepatology* (Amsterdam) 11: 374-380.
- Rey, F., G. Donker, B. Spire, C. Devaux & J. C. Chermann, 1991. Selection of an HIV-1 variant following anti-CD4 treatment of infected cells. *Aids-Forschung* 6: 243-246.
- Righthand, V. F., 1991. Transmission of viral persistence by transfection of human cultured cells with RNA of a persistent strain of echovirus 6. *Microbial Pathogenesis* 11: 57-66.
- Robert-Guroff, M., M. S. Reitz, W. G. Robey & R. C. Gallo, 1986. In vitro generation of an HTLV III variant by neutralizing antibody. *Journal of Immunology* 137: 3306-3309.
- Saag, M. S., B. H. Hahn, J. Gibbons, Y. Li, E. S. Parks, W. P. Parks & G. M. Shaw, 1988. Extensive variation of HIV-1 in vivo. *Nature* (London) 34: 440-444.
- Salinovich, O., S. L. Payne, R. C. Montelaro, K. L. Hussain, C. J. Issel *et al.*, 1986. Rapid emergence of novel antigenic and genetic variants of equine infectious anaemia virus during persistent infection. *Journal of Virology* 57: 71-80.
- Salvato, M., P. Borrow, E. Shimomaye & M. B. A. Oldstone, 1991. Molecular basis of viral persistence: A single amino acid change in the glycoprotein of lymphocytic choriomeningitis virus is associated with suppression of the antiviral cytotoxic T-lymphocyte response and establishment of persistence. *Journal of Virology* 65: 1863-1869.
- Simmonds, P., P. Balfe, C. A. Ludlam, J. O. Bishop & A. J. L. Brown, 1990. Analysis of sequence diversity of hypervariable regions of the external glycoprotein of human immunodeficiency virus type 1. *Journal of Virology* 64: 5840-5850.
- Stanley, J., L. M. Bhaduri, U. Narayan & J. E. Clements, 1987. Topographical rearrangements of visna virus envelope glycoprotein during antigenic drift. *Journal of Virology* 61: 1019-1028.
- Staskus, K. A., L. Couch, P. Bitterman, E. F. Retzel, M. Zupancic, J. List & A. T. Haase, 1991. *In situ* amplification of visna virus DNA in tissue sections reveals a reservoir of latently infected cells. *Microbial Pathogenesis* 11: 67-76.
- Stoye, J. P. & J. M. Coffin, 1985. Endogenous retroviruses, volume 2 in *RNA Tumor Viruses*, edited by R. Weiss, N. Teich and J. Coffin. Cold Spring Harbor Laboratory, New York.
- Sunaina, V., V. Kishore & G. S. Shekhawat, 1989. Latent survival of *Pseudomonas solanacearum* in potato tubers and weeds. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* 96: 361-364.
- Tangy, F., A. McAllister, C. Aubert & M. Brahic, 1991. Determinants of persistence and demyelination of the DA strain of Theiler's virus are found only in the VP1 gene. *Journal of Virology* 65: 1616-1618.
- Tersmette, M., R. E. Y. De-Goede, J. K. M. Eeftink-Schattenkerk, P. T. A. Schellekens, R. A. Coutinho, J. Goudsmit, J. M. A. Lange, F. De-Wolf, J. G. Huisman & F. Miedema, 1989. Association between biological properties of human immunodeficiency virus variants and risk for AIDS and AIDS mortality. *Lancet* 1: 983-985.
- Tersmette, M., R. A. Gruters, F. De-Wolf, R. E. Y. De-Goede, J. M. A. Lange, P. T. A. Schellekens, J. Goudsmit, H. G. Huisman & F. Miedema, 1989. Evidence for a role of virulent human immunodeficiency virus (HIV) variants in the pathogenesis of acquired immunodeficiency syndrome: Studies on sequential HIV isolates. *Journal of Virology* 63: 2118-2125.
- Terwilliger, E. F., E. Langhoff, D. Gabzuda, E. Zazopoulos & W. A. Haseltine, 1991. Allelic variation in the effects of the nef gene on replication of human immunodeficiency virus type 1. *Proceedings of the National Academy of Sciences of the United States of America* 88: 10971-10975.
- Vartanian, J. P., A. Meyerhans, B. Asjo & S. Wain-Hobson, 1991. Selection, recombination, and G to A hypermutation of human immunodeficiency virus type 1 genomes. *Journal of Virology* 65: 1779-1788.
- Vasek, F. C., 1980. Creosote bush: long-lived clones in the Mohave desert. *American Journal of Botany* 67: 246-255.
- Whetstone, C., J. Miller, D. Bortner & M. Van-Der-Maaten, 1989. Changes in the restriction endonuclease patterns of four modified-live infectious bovine rhinotracheitis virus (IBRV) vaccines after one passage in host animal. *Vaccine* 7: 527-532.
- Wolfe, M. S., 1985. The current status and prospects of multiline cultivars and variety mixtures for disease resistance. *Annual Review of Phytopathology* 23: 251-273.
- Wolfs, T. F. W., J. J. De-Jong, H. Van-Den-Berg, J. M. G. H. Tijnagel & W. J. A. Krone, 1990. Evolution of sequences encoding the principal neutralization epitope of human immunodeficiency virus 1 is host dependent, rapid, and continuous. *Proceedings of the National Academy of Sciences of the United States of America* 87: 9938-9942.
- Wolfs, T. F. W., G. Zwart, M. Bakker, M. Valk, C. L. Kuiken & J. Goudsmit, 1991. Naturally occurring mutations within HIV-1 V3 genomic RNA lead to antigenic variation dependent on a single amino acid substitution. *Virology* 185: 195-205.
- Yoshikawa, N. & R. H. Converse, 1990. Strawberry pallidosis disease: Distinctive double stranded RNA species associated

- with latent infections in indicators and in diseased strawberry cultivars. *Phytopathology* 80: 543-548.
- Zurbriggen, A., C. Thomas, M. Yamada, R. P. Roos & R. S. Fujinami, 1991. Direct evidence of a role for amino acid 101 of VP-1 in central nervous system disease in Theiler's murine encephalomyelitis virus infection. *Journal of Virology* 65: 1929-1937.
- Zweig, M., K. P. Samuel, S. D. Showalter, S. V. Bladen, G. C. Dubois, J. A. Lautenberger, D. R. Hodge & T. S. Papas, 1990. Heterogeneity of Nef proteins in cells infected with human immunodeficiency virus type 1. *Virology* 179: 504-507.