SESSION V

CORRELATIONS AND CONCLUSIONS

The meaning of persistent infections in nature

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Viruses that cause persistent infections maintain themselves more effectively in nature than those causing acute and limited infections. There is a tendency for persistent viruses to evolve towards a state of minimal pathogenicity in the host. Vertical transmission, with integration of viral into host genome, represents the state of perfect parasitism.

I am going to use the word persistent to refer to infections in which the virus remains in the body for long periods, often for life, and is always demonstrable in some form or other. This includes infections with many herpes-type viruses (herpes simplex, varicella-zoster, Epstein-Barr (EB) virus, cytomegalovirus), adenoviruses, leukaemia viruses, and arenaviruses. It might be thought that certain viruses, such as the picornaviruses, inevitably cause acute and limited infection, but even these can cause persistent infections. Theiler's virus (mouse polio) classically behaves in this way in the central nervous system, and foot-and-mouth disease virus has been shown to cause persistent infection in horses. Viruses may persist in the fully infectious state, in a non-infectious form in which viral antigens only are formed, or as a mere nucleic acid presence in the cell. Persistent infections tend to be minimally damaging to the host, and are not important causes of chronic ill health or mortality. Tissues infected include especially the central nervous system (certain herpes viruses), lymphoid tissues (EB virus, adenoviruses), and parts of the body from which viruses can be shed to the exterior, such as the salivary glands (cytomegalovirus), kidney tubules (polyoma virus), and mammary glands (mammary tumour virus). Murine leukaemia and chicken leucosis viruses infect all cells in the body.

The persistent viruses discussed here will include especially the ones carried by rodents, and some of these infect man. It may be noted that although many animals have found their way into English phrases, some having affectionate connotations (" my little lamb ", " ducky ") and some referring to an obvious feature of an animal (" fat " or " filthy pig "), there is one animal that is used almost entirely in derogatory or abusive phrases. It is surely no accident that it is the rat. The Egyptians and Phrygians deified this animal, but human experiences with diseases such as plague and typhus have perhaps been responsible for the downfall of the rat. Many other human infections are derived from rats and other rodents, including of course the virus infections we have been discussing at this meeting.

The meaning of persistent infections in nature will be discussed under 4 headings. Although few of the concepts are new, and others may be self-evident, they are nevertheless worth consideration.

1. *Persistent infections maintain themselves more readily in nature*

Acute infections (smallpox, measles, or influenza) remain in the infected individual for at the most a few weeks, and must then find a new susceptible host. If there is none, the infection dies out locally until the appearance of fresh susceptibles and the reintroduction of virus into the community. Studies of island communities (1) have shown that measles needs a minimum population of 500 000 if it is to maintain itself by successive infection of susceptible individuals. It is only recently that human populations have become large enough to support continuously transmitted infections of this type, and in paleolithic times, when men lived in small isolated groups of 30–50, such infections could not have existed. Persistent infections, on the other hand, are not so limited in this way. Chickenpox can maintain itself indefinitely in a population of less than 1000, because after infecting susceptible hosts the virus persists in dorsal root ganglia. Many years later, when a fresh group of susceptibles has appeared, an occasional old person develops zoster, and the virus shed from these vesicles can once again produce chickenpox in the community. Other persistent vi-

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ruses are shed more or less continuously from the body for long periods, such as arenaviruses in the urine and saliva of rodents and polyoma virus in mouse urine.

Many animals live as man used to, in small, rather isolated groups, and the most successful viruses tend to be those that persist. This is illustrated in recent studies of isolated human communities. The virus infections that were found most commonly by serological testing in 7 isolated Indian tribes on the periphery of the Amazon basin (2) were the persistent viruses herpes simplex, EB virus, cytomegalovirus, varicella, and hepatitis B. Those that were totally absent from one or more tribes included nonpersistent viruses, such as measles, mumps, rubella, influenza, and parainfluenza viruses. Persistent nonviral infections, such as typhoid, leprosy, and typhus, would also tend to maintain themselves in smaller populations.

Persistent infection is significant for the virus and for its maintenance in nature only in so far as it encourages or is necessary for the spread of infection between individuals. This includes the viruses transmitted via the germ line (see below), which must persist at least in germ cells. Apart from this, if there is no shedding to the exterior and to fresh hosts, persistence is irrelevant both for the virus and for its maintenance in nature. The viruses present in diseases such as subacute sclerosing panencephalitis (SSPE) and progressive multifocal leukoencephalopathy (PML) come into this category.

Even in acute virus infections, the period of shedding may be of critical importance. In the Australian rabbit, pathogenic strains of myxoma virus were replaced by nonpathogenic strains, because the virus-rich skin tumours of the nonpathogenic strains persisted for longer periods and gave greater opportunity for mosquito-borne spread to fresh hosts (3). Rabies is another example of a disease in which a prolonged period of shedding is important for the maintenance of the infection in nature. If rabies virus killed the European fox within a day or two there would be less opportunity for salivary spread of the infection and the disease might die out in Europe.

There is no evidence that the arenaviruses we are discussing persist for any great length of time in man, the unnatural host. I believe that by far the most important feature of the pathogenesis of Lassa fever virus in man is the limited person-to-person transmission. Person-to-person transmission is not a feature of any other arenavirus infections, and no more than one or two transfers appear to take place with Lassa fever. As long as this remains so, Lassa fever can never be more than an insignificant cause of human illness or death. If, however, there were a change in the virus, so that it spread effectively from person to person, an " Andromeda strain " situation would easily arise. The most important and least controllable method of microbial spread in crowded human communities is by the respiratory route, and the limited person-to-person transmission of Lassa virus perhaps depends on the restricted extent of virus replication in the throat and respiratory tract, as a result of which rather limited amounts of virus are shed into the air (most patients cough). A change in the pattern of virus replication in the throat and respiratory tract might be of greater significance for man than an understanding of the disease process itself.

2. *The pathogenicity of persistent viruses*

Persistent virus infections cannot be too pathogenic because if persistence is to be of selective advantage for the virus, the host must survive, remain in the community, and either shed the virus or otherwise hand it on to uninfected individuals. Ideally, from the virus point of view there should be little or no inconvenience to the host, as with chickenpox in man. If the host is seriously affected, selective pressures will eventually eliminate the host together with the virus. There must, in fact, be a balanced pathogenicity if the virus-host relationship is to be stable and last for at least a few thousand years. A minimum amount of pathogenicity may be needed for virus shedding, as with herpes simplex lesions in the oropharynx, but viruses evolve with great rapidity and strains with the least possible pathological effects are likely to emerge as the host-parasite balance settles down in evolutionary terms. Of course, at any given time there will be many animal viruses that have not reached this equilibrium, especially with infections of man and certain domestic animals in which the host species has been undergoing an astronomical increase in numbers, offering golden opportunities to microorganisms that spread efficiently in crowded communities. A severe infection may cause disastrous epidemics and decimate the host species before settling down, as was seen in the example of myxomatosis in the Australian rabbit, where both virus and host evolved towards a more balanced pathogenicity. The same phenomenon may be occurring today with cholera in

man as the less pathogenic and more readily transmitted El Tor strain spreads and flourishes.

But although we can identify associations between persistent virus and host that have already reached a degree of stability, with minimal pathogenicity, these persistent infections are not entirely harmless. In the first place, viral antigens are often continuously released into the circulation in the infected host, as a result of which immune complexes may be formed and cause diseases such as glomerulonephritis, as we have heard. This is a serious matter in certain strains of mice carrying lymphocytic choriomeningitis (LCM) virus, but is of little consequence in mice carrying leukaemia or lactic dehydrogenase (LDH) viruses. Second, the persistent viral genome itself sometimes causes tumours, albeit with low frequency and with no benefit to the virus, as in the murine, bovine, and feline leukaemias. Third, the infection itself can light up again and cause a fresh episode of the disease. This is generally a necessary step for renewed virus shedding to the exterior, as in psittacosis in birds and in herpes simplex and zoster in man. In man, such shedding also occurs as an unfortunate consequence of immunosuppression induced by the physician (warts or cytomegalovirus disease in renal transplant patients) or by an immunosuppressive disease (warts or tuberculosis in patients with Hodgkin's disease). Fourth, there may be other poorly understood deleterious effects, such as the reproductive failure found in some strains of mice carrying LCM virus (6) and also in *Calomys callosus* carrying Machupo virus. Given time, selection will operate in persistent infections to minimize the undesirable " side-effects " by changing the genetic constitution of both host and virus.

One conclusion from the work with LCM virus in different laboratory strains of mice is that relatively small genetic changes in the host may result in important changes in pathogenicity or in virus carriage. It would be interesting to know whether different local strains of *Mastomys natalensis* differ in their susceptibility and ability to carry Lassa virus.

3. *Meaning of persistent infection with vertical transmission via the germ line*

Vertical transmission strictly includes transfer from parent to offspring via milk, urine, and contact, but this must be distinguished from transmission via the ovum (murine leukaemia, LCM) or sperm (mammary tumour virus). If the fertilized egg and all embryonic and adult cells are infected, and if all members of the species are infected, then horizontal transmission becomes irrelevant. It is no longer necessary that infectious virus or viral antigen should be produced, as long as a nucleic acid presence is maintained in the species. What matters for virus success in evolutionary terms is that the virus maintains itself in some form or other. Infectious virus therefore ceases to be produced, in much the same way that vision eventually disappears in cave-dwelling animals, or the ability to take a swift blood meal and thus escape the attentions of the host is reduced in colonized (artificially fed) mosquitos (4). Indeed if horizontal transmission is really quite irrelevant, there is every reason why less and less of the viral genome should be carried in the host. There is no greater success for a microorganism than its continued presence in all members of the host species, and from this point of view it is of no consequence whether it is present in the form of infectious virions or a mere length of nucleic acid.

But the position is more complicated than this. A virus transmitted via the germ line is less secure than might appear at first sight if occasional individuals lose the virus and the viral genome by genetic or other mechanisms. If this loss gives the individual some slight selective advantage (for instance, by escaping the reproductive inefficiencies seen in certain rodents carrying arenaviruses), then a virus-free strain of individuals will emerge, and the virus, because it is genetically defective, will never be able to recolonize the species. Also, in rodents at least, where there are great population crashes in which only a few individuals survive, the chance of the subsequent recolonization of territory by a virus-free individual is higher. A virus that is transmitted exclusively in the germ line and becomes irreversibly defective is also at a disadvantage because there is no opportunity to colonize different species of animals, other than those arising in evolution from the carrier species.

For reasons such as these, perhaps, the viruses we know about are still present in infectious form for much of the time. Murine leukaemia virus, although present only as genome and possibly as antigen in the embryos of many mouse strains, is fully infectious under other circumstances. Feline leukaemia virus (5) is transmitted horizontally by the shedding of infectious virus in saliva, germ-line transmission being either sporadic or non-existent. This could represent a relatively recent acquisition of the virus by cats, a possibility that is also suggested by the harmful effects (anaemia, immunosuppression, re-

tarded growth, glomerulonephritis) seen in infected cats.

Nevertheless, once the infection is transmitted in the host genome and most individuals are infected, it seems inevitable that the virus will tend to become " vestigial ", and present only as a defective viral genome. One consequence of this is that the virus is then unlikely to prevent superinfection of the species with other viruses of the same type. Mice are susceptible to infection with laboratory strains of murine leukaemia virus even though they carry complete murine leukaemia virus genomes. If this is so, then many viral genomes are likely to have been incorporated into the host genome during the course of evolution, as suggested by the work of Todaro & Huebner (7) and others. At times they may be randomly lost as a chance result of genetic changes, but at any given time many will be present. Viruses evolve with great rapidity, and if they tend to reach the stage which we must call perfect parasitism (transmission via the host genome) without great difficulty, it is to be expected that at any given time many viral genome fragments will be present in the host genome. Both the murine leukaemia-sarcoma viruses and the chicken tumour viruses exist in more than one form, with defective " helper " viruses present in the same individual, and complex control by host genetic factors. Conceivably this reflects the continued entry of viruses of this group into the host genome.

4. *Possible advantages to the host of persistent viral infections*

One possible advantage of persistent infections is that they often maintain effective resistance to reinfection by constantly stimulating immune responses. Many viruses, including mumps, chickenpox, EB virus, and poliovirus cause more serious diseases when primary infection takes place in adults. Long-term immunity to viruses such as mumps and polio can be maintained without persistence, but in the case of viruses such as EB and chickenpox persistence perhaps gives more effective, lasting immunity. Viruses that initiate persistent infection in early life can be said to protect against the more serious consequences of primary infection in later life.

Persistent arenaviruses in their rodent hosts certainly protect against infection in later life, but here it is less clear that infection in later life is in fact more severe. Newborn mice infected with LCM virus generally develop less severe disease than do adult mice infected intracerebrally, yet it is difficult to imagine a more unnatural route of infection than the favourite intracerebral one. Adult mice infected by a more natural extraneural route, such as the foot, suffer no harmful effects, except for a temporarily swollen foot.

The question may be asked (but not answered) whether persistent viral genomes are of any value to the host by coding for possibly useful proteins and providing an additional source of genetic variation. In an analogous situation the beta phage carried by *Corynebacterium diphtheriae* codes for the production of the diphtheria toxin, and enteric bacteria derive undoubted advantages from carrying the plasmids or episomes that produce colicines and resistance to antibiotics.

There is one other conceivable advantage to the host of a persistent tolerated virus infection, although this, too, is speculation. If there is an animal population (species or subspecies) related to the natural host that overlaps geographically and competes with the host, then this other population may also become infected. The infection may well be more pathogenic in the unnatural host and perhaps transmitted vertically. In this way the infection would confer a selective advantage on the original persistently infected host. This is perhaps likely to occur with arenaviruses spreading between geographically overlapping species of rodents. Even a reproductive inefficiency in the unnatural host could give the original host an advantage. In much the same way, the European (*Oryctolagus*) rabbits introduced into South America were infected by myxoma virus originating from the natural and harmlessly infected South American (*Sylvilagus*) host. Presumably this had some influence in controlling the numbers of the European competitor. Also, the cattle, sheep, and horses introduced into Africa were exposed to viruses, such as bluetongue, rinderpest, and African horse sickness, that had evolved with the great herds of African ungulates. In the natural host they caused little harm, but there was a devastating effect on the domesticated newcomers.

As far as persistent infections go, the situation would be analogous with that of the primate herpes viruses such as *Herpesvirus saimiri* and *Herpesvirus ateles*, where the naturally and persistently infected host suffers no ill effects, whereas in other species the virus induces lymphomas. It is not suggested that in this case infection of another primate species takes place and is of any significance under natural circumstances. Nor is there any evidence that rodents persistently infected with the arenaviruses are at any

advantage for reasons of this sort. It would nevertheless by very interesting to study the matter. For instance, does LCM virus establish persistent infection in *Calomys callosus* or in *Mastomys natalensis*? Does Machupo virus establish persistent infection in *Mus musculus*, and if so, what are the pathological consequences? Can a given host carry two arenaviruses, for instance LCM and Machupo in *Mus musculus* or *Calomys callosus*, or is there mutual exclusion?

RÉSUMÉ

SIGNIFICATION DES INFECTIONS PERSISTANTES DANS LA NATURE

Que le virus soit transmis sans interruption, activé par intermittence ou intégré dans le génome de l'hôte, les infections persistantes se maintiennent plus aisément dans la nature. Comme les autres infections, elles tendent vers un état équilibré de pathogénicité minimale; les infections qui n'ont pas encore atteint cet état d'équilibre seraient d'origine relativement récente. On ignore si une quelconque infection persistante confère un avantage à l'hôte; on pourrait notamment concevoir qu'elle lui permette d'infecter une population animale concurrente, plus sensible.

Lorsque le virus est intégré dans le génome de l'hôte, ce qui représente l'état de parasitisme parfait, il tend à devenir de plus en plus défectif. Il peut également arriver que le génome viral disparaisse. Il n'est pas impossible qu'il y ait une évolution constante des virus dans ce sens.

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DISCUSSION

MAIZTEGUI: In view of the overlapping you mentioned, and since we have found simultaneous activity of Junin and LCM viruses in human beings and rodents of the endemic area of AHF, one wonders which one of them will finally give the advantage to its respective hosts, a matter of considerable importance in public health because of the high mortality of AHF.

MIMS: I presume that there would be some interaction, but I would like to know whether, in fact, these two viruses can co-exist in the same individual host, or whether it was simply that the two hosts were living together on a micro-ecological scale and overlapping physically in their habitat.

WOODALL: You have also raised the horrifying possibility that *Mastomys* might not be the final reservoir of Lassa virus, but only the indicator, with another host harbouring the virus cryptically.

MIMS: I merely illustrated a point that has been made earlier, that the study of the rodent host of the arenaviruses is in a rather primitive state.