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SPECIAL ARTICLE

Virology research and virulent human pandemics

SUMMARY

The possibility that a devastating human pandemic could arise, causing massive loss of human life, is discussed. Such a major threat to the human species is likely to be a virus, and would spread by the respiratory route. It need not necessarily cause massive loss of life, but if it caused serious illness or incapacity it would still have a major impact. A possible source is from an existing respiratory pathogen, but it would more probably arise from an infection that is maintained in an arthropod or vertebrate host, but which at present either does not infect humans, or if it does it fails to be effectively transmitted between them. More research should therefore focus on the pathogenetic factors and the viral determinants that promote respiratory transmission.

INTRODUCTION

Great plagues have at times influenced, and possibly determined, the course of history [1]. Armies have been defeated by typhus rather than by the enemy, and the plague, for instance, killed about a third of the population of England in the mid 14th century, with a major effect on the country's ambitions in Europe and on patterns of rural life. The effects of cholera, tuberculosis, and smallpox in 19th century England were still serious, but less devastating. Some of these infections, however, show greater mortality in previously unexposed populations, as in the case of smallpox in the Incas and Aztecs, or tuberculosis in the peoples of Africa.

More recently, the 1918–19 influenza pandemic is estimated to have killed 20 million people, and by the end of this century at least 10–20 million may have died of AIDS. Yet these infections have not had a catastrophic effect on our species. In spite of the great suffering caused, less than 1% of the world's population was affected.

It can be argued that for crowded humanity, the threat of infectious disease is far greater than that from cancer, heart disease, accident, or war. Could a more serious infection arise and disrupt life on the planet by killing or seriously incapacitating a third or a half of the world's population? This paper discusses the probable nature and source of such a global pandemic or 'Andromeda strain' of microbe. An Andromeda strain (from the 1965 novel 'The Andromeda Strain' by Michael Crichton) is a hypothetical new type of microbe that causes massive destruction of human life. Its impact would depend on its transmissibility, and transmissibility is a neglected subject in microbiology.

TRANSMISSION AND THE RESPIRATORY ROUTE

The transmission of infectious disease is a neglected subject [2]. To a large extent current research is concerned with the microbial gene products that cause

disease, and the host cells and cytokines that mediate resistance and recovery from infection. HIV has alerted us to our ignorance about mechanisms of transmission. Practical questions have included whether infection can be acquired from a mosquito, from a shared toothbrush or communion cup, and the observation that the uncircumcized are more susceptible to infection [3] is unexplained.

Most modes of transmission can be interrupted by appropriate public or personal health measures. Clean water, food hygiene and good sewage disposal for faecal–oral infections; condoms and treatment of infected carriers in the case of sexually transmitted infections; the often difficult control of vectors and reservoirs for arthropod-borne infections and zoonoses.

The respiratory route is different firstly because infection spreads so rapidly in crowded communities. The average human inhales about 10000 litres of air daily, and we constantly and unavoidably inhale each other's respiratory discharges. Thus everyone sharing the same airspace is likely to be exposed. In contrast to sexually transmitted or faecal–oral infections a single human may by respiratory spread infect a score of others in a day. The incubation period is often short so that the new cases appear within a week. Air travel is common and the infection soon becomes worldwide. A life-threatening respiratory epidemic easily brings out that mediaeval fear of contagion that lies in all of us, as was apparent in the recent outbreak of pneumonic plague in Surat, India [4].

The respiratory route is also different because it is difficult to interrupt this type of transmission [5, 6]. In the absence of cheap, effective vaccines the only methods are isolation of infected individuals, the use of masks, air flow control, air changes (dilution effect), and the filtering and u.v. irradiation of air. Isolation is impossible unless the infection is both rapid and severe; if not the individual will circulate in the community and infect others. Masks are reassuring but generally ineffective, and full protection is only obtained by heavy, cumbersome respirators. Laminar flow systems, filters, and u.v. irradiation are possible in specialized areas like schoolrooms, laboratories, hospitals, but impracticable and expensive for public spaces. The effect of air flow and filtering is seen in the Alaska, 1977, outbreak of influenza aboard a commercial aircraft. The aircraft was delayed on the ground for 3 h because of engine failure and most people stayed on the plane. It was cold, so the doors were kept closed much of the time, but the ventilation system was not working. During this short period one passenger, suffering from influenza A (H3N2) infection, who was unwell and had a cough, transmitted the infection to 38 (72%) of the other 54 passengers [7].

Therefore it is suggested that a devastating human infection would be transmitted by the respiratory route. It would probably eventually die out if it killed too many people, but the damage would already have been done. It would also die out in small, isolated populations [8], although nowadays there are few of these.

THE NATURE OF THE INFECTIOUS AGENT

The infectious agent is likely to be a virus, but not necessarily so. There are hundreds of different viruses in circulation that infect non-human vertebrates, some of them transmitted by arthropods. In recent years new and virulent human

infections from this source have nearly always been viral (Ebola, Marburg, Lassa, hantaviruses), and as humans in larger numbers colonize the remotest parts of the earth, encounters with wildlife virus cycles become more frequent. Also, while non-viral infections can generally be controlled by antimicrobial drugs, at present this is not the case for viral infections.

THE SOURCE OF THE INFECTION

Most new human infections arise from non-human vertebrate reservoirs and are often transmitted by arthropods. This is true for hantaviruses, Lassa fever, monkeypox, and presumably (although the source is not identified) for Marburg and Ebola viruses. Other infections that come from this source include psittacosis, typhus, leptospirosis, brucellosis, bubonic plague, anthrax, trypanosomiasis, and yellow fever. Other infections, such as Legionnaire's disease or cholera, arise from environmental sources.

One striking characteristic shared by all these infections is that none of them is effectively transmitted from person to person. Ebola virus spreads from person to person, especially in hospitals, by direct or indirect contact with infected body fluids, but there is no evidence for airborne transmission [9]. Nurses, physicians or pathologists have acquired Lassa fever virus from infected patients [10], and monkeypox virus has been transferred through as many as four cycles in humans before dying out [11]. However there is no continued human chain of infection, and fresh cases depend on repeated exposure to the source. These zoonotic and arthropod-borne infections are often virulent in humans, but without continued person to person (respiratory) transmission they remain focal, sporadic, and can never pose a major threat to our species.

THE REQUIREMENTS FOR AN 'ANDROMEDA STRAIN'

The infectious agent, probably a virus and maintained in an arthropod or animal host system, must infect humans and develop the capacity for respiratory transmission. A virus that trod this pathway 1000–2000 years ago and is transmitted directly from person to person by this route is measles, which probably arose fron rinderpest in cattle. Smallpox also arose in this way, although the exact source is uncertain. HIV (from African primates) has developed the capacity for sexual transmission between humans, and has become one of the most important infections in the world, but in its present form it is not a serious threat to our species.

The microbe must also be virulent, causing a frequently fatal or incapacitating disease. This property may bear no relation to transmissibility. Classic studies were carried out with different strains of influenza A virus in mice [12]. The Jap 305 strain, after intranasal inoculation, was transmitted to 62·5% of contact mice, whereas the PR8 and NWS strains were transmitted to only 5–8%. Yet lung virus titres were the same, and transmissibility was not connected with virulence. The mechanism for the different transmission rates remains unknown. The Jap 305 strain was not more stable in air, and there were no differences in the amount needed to infect mice by aerosol. However, when exhaled air samples obtained from impinger flasks were assayed, those from Jap 305-infected mice contained significant amounts of virus whereas no virus could be recovered from NWS-

infected mice. This difference in shedding from the lungs could involve differences in neuraminidase activity.

The main requirement for respiratory transmission is that large enough quantities of the microbe are shed from the respiratory tract, preferably to the accompaniment of coughing or sneezing, and preferably allowing the individual to circulate in the community for a day or two, thereby facilitating spread of the infection before the onset of illness.

The requirement for respiratory transmission is best analysed in terms of the pathogenesis of the disease, and it will be assumed that it is caused by a virus.

A. The virus must localize in respiratory epithelium

Many viruses transmitted by the respiratory route merely grow and spread across the mucosal surface after initial infection, and are shed after a few days without invading deeper tissues. For the most part (rhinoviruses, non-pandemic influenza, parainfluenza viruses and coronaviruses) they do not cause serious illness. It is the viruses that cause generalized infection, whatever the initial route of infection, that have greater pathogenic potential. They have a viraemic phase and cause disease after invading tissues such as the brain, liver or the immune system. If one of these viruses is to be transmitted by the respiratory route it must leave the blood and localize, like measles does (and smallpox did), in the respiratory tract, where it can multiply and be discharged to the exterior.

What are the pathogenetic factors involved? The circulating virus has to localize in capillaries in the lung or nasal mucosa, and traverse the endothelium to reach subepithelial or alveolar sites. It must then enter epithelial cells from the basal surface.

Almost nothing is known of the mechanism by which circulating viruses localize in particular target organs and tissues [13]. The localization of mumps virus in salivary glands, of polioviruses in nervous tissue, or (more to the point) of measles or rubella viruses in respiratory epithelium, remains a mystery. But information is available about the localization of circulating immune cells in the vascular bed of different lymphoid organs. Homing molecules on the surface of lymphocytes bind to molecules (vascular addressins) on vascular endothelium in peripheral lymph nodes, in gut-associated lymphoid tissue or in inflamed tissue. Different vascular addressins in these sites enable the different types of lymphocyte to recirculate through appropriate tissues [14]. In other words different molecules are displayed on the endothelium of different tissues. Might some of these act as 'viral addressins'? Does measles, or a measles-infected leucocyte, bind to a defined molecule on the lumenal surface of lung capillaries?

If the virus is arrested in lung or nasal capillaries it must now grow across or be passively transported across endothelium in the direction of neighbouring epithelium. Cells arranged in sheets show biochemical polarization, which has an effect on viral entry and exit [15, 16]. Some viruses only enter or are only shed from the free apical surface of the cell whereas others are restricted to the basolateral surfaces. If the Andromeda strain grows across endothelial cells and these are polarized, then it must enter the apical surface facing the lumen, and be shed from basolateral surfaces. There is then a basement membrane to be traversed, followed by the problem of entering the polarized epithelial sheet from

the basal surface. After replication it would need to be liberated from the free surface.

These requirements are illustrated by the pathogenesis of measles. Infection is initiated somewhere in the respiratory tract, but the virus then fails to spread laterally on the epithelial surface and instead invades deeper tissues to establish a systemic infection. At a later stage, and by unknown mechanisms, circulating virus relocalizes in the respiratory tract, where there is extensive replication. At this stage, measles virus presumably does spread laterally in epithelial cells, and it is worth asking why this does not occur immediately after infection. Perhaps initial entry is into a special cell type from which progeny is not released onto the apical surface. If so, such cells must be fairly common because infection seems to be initiated by a small amount of virus. Answers to questions like this might help identify factors determining the vital phenomenon of epithelial invasion and spread when blood-borne viruses localize in the respiratory tract.

The importance of this particular step in pathogenesis is illustrated by hantaviruses [17–19]. Infection is acquired from rodents, and virus spreads systemically in the human host and infects vascular endothelium. The Korean hantavirus localizes in kidneys, causing a life-threatening renal disease (Korean hemorrhagic fever), whereas the SW American strain localizes in the lung and gives rise to a severe pulmonary syndrome. The widespread infection of lung capillaries causes fluid outpouring into the lungs; 26 people died in recent outbreaks in SW USA. But the virus fails to spread from capillary endothelium to the nearby respiratory epithelium. We know nothing of the reasons for this restriction. If hantaviruses are capable of infecting respiratory epithelium, it is this restriction that prevents it from becoming a major respiratory pathogen. Non-viral infections where the lung is involved but there is a failure to invade respiratory epithelium include psittacosis, Q fever and Legionnaires disease.

B. The virus must productively infect epithelial cells and spread efficiently from cell to cell, either directly or after being shed onto the respiratory surface

Many factors determine cell susceptibility. In the first place it depends on the cell bearing specific receptors for the microbe [20, 21]. Once in the cell, productive replication may depend on the temperature. Rhinoviruses fail to spread to the lower respiratory tract largely because it is too warm there, their optimum growth being at the lower temperature of the nasal mucosa (22). The state of differentiation of the cell can be a critical factor; this is so for epidermal cells (wart viruses), as well as for neurones, hepatic cells, lymphocytes, or macrophages in the case of other viruses [23, 24]. A cell may fail to replicate the virus because it fails to recognize viral enhancer sequences, as shown by the highly restricted expression of viral genes in transgenic mice. JC papovavirus genes, for instance, are only expressed in oligodendrocytes [25], and these are the cells involved in progressive multifocal leucoencephalopathy, caused by this virus.

Even if viral replication is initiated, maturation may depend on the presence in the cell of the specific proteases needed to cleave viral polypeptides. Growth of parainfluenza and influenza viruses in cells, and the spread through the body and infection of different tissues by avian influenza viruses, have been shown to be determined by the presence of the necessary cell proteases [26].

Finally, the virus, unless it causes the briefest of 'hit and run' infections where replication and shedding is completed within a few days, will have to evade the hosts immune and inflammatory responses. This will give time for replication and transmission even though the microbe is eliminated from the body soon afterwards. Systemic viruses, in particular, are proving to have gene products that interfere with immune and inflammatory responses [27]. These include interference with the activation and action of complement, the prevention of cytotoxic T cell recognition and destruction of the infected cell, and evading interferon. Interferons have long been thought to be potent host defences against virus infection, especially hit-and-run respiratory infections where instantly available defences are more likely to be effective than relatively slowly induced immune responses. The fact that so many viruses, including influenza [28] have developed strategies either for not inducing or for being insusceptible to interferons, reflects their significance in host defence.

C. Multiplication of the virus in or on respiratory epithelial cells must be extensive enough to ensure adequate shedding to the exterior

Enough cells must be infected and sufficient progeny produced in each cell. Surprisingly little is known about the factors determining viral yield from cells, or of the differences in yield between different cell types. The amount of virus shed varies of course with the stage of the infection, but also, for unknown reasons, peak titres differ from individual to individual [29, 30] and some people are better transmitters than others. For transmission, the amounts shed have to be considered in relation to microbial stability in droplets, and the host's susceptibility as indicated by the minimal dose needed to infect by the respiratory route.

Mere shedding does not necessarily ensure transmission. The infection must also be effectively disseminated. Important factors include the induction of respiratory secretions, and their fluidity. Face to face contact after coughing or (preferably) sneezing provides good opportunities for respiratory spread. This does not call for great viral resistance to drying, thermal inactivation, or u.v. light, although these properties may be important in the case of rhinoviruses which can be transmitted by fingers as well as aerosols [31, 32].

Virus stability becomes an issue when transmission takes place also over longer distances or over longer periods of time, e.g. entering a room 10 min after a virus-laden sneeze. Measles for instance, is transferable via droplet nuclei less than 4 μ in diameter, which remain suspended in the air after formation [33]. In classic studies of measles in schools, epidemics were controlled by u.v. irradiation of air [34]. Varicella can spread over medium distances, such as between rooms and wards in hospitals [35], and foot and mouth disease provides an extreme example. An outbreak of this disease in the Isle of Wight, England, was caused by the virus travelling in air for 50 miles across the English Channel from France and retaining its infectivity [36].

Transmission also depends on the number, density and behaviour of the host. In crowded communities, whether humans in cities, chickens in batteries, or horses at race meetings, there are great opportunities for the spread of infection. For instance influenza epidemics occur late in the winter [37], and to a large extent this

is because people are then indoors, often with poor circulation of air. An additional indoor factor is that when air is heated the relative humidity falls to 40% or less, at which respiratory viruses are generally more stable [38]. A 2–3 °C fall in outside air temperature is followed a few days later by an increased incidence of respiratory infections. If a new strain of influenza virus arrives in the country in the summer it must wait until the winter before it can cause an epidemic.

EXAMPLES OF POSSIBLE SOURCES

Could an existing human pathogen develop the capacity for respiratory transmission? Viral possibilities include HIV, Lassa, Ebola, Marburg, rabies, hantaviruses, and there are also bacteria such as the Rickettsiae, chlamydia, leptospira, brucella. The possibility of a switch to respiratory transmission in the case of Lassa fever virus has been discussed earlier [39]. The virus is present in the throat of infected patients, but perhaps only in small quantities. However, when acquired from its natural rodent host in West Africa, it causes only a mild febrile disease in most individuals [40]. Even HIV appears to be able to infect epithelium, albeit inefficiently, by uncertain mechanisms, and with low yield [41]. But the virus is inherently variable in its envelope antigens, and a change in tropism is always a possibility. The fact that an individual can simultaneously be infected by more than one virus strain and that these strains can 'hybridize' [42] provides additional opportunities for generation of an HIV variant that spreads by the respiratory route.

Nature is always providing opportunities for such a shift in transmission to take place. Many of the hundreds of different arthropod-borne viruses are constantly being introduced into humans, and any variants capable of spreading directly from person to person would rapidly be selected out.

Monkeypox, naturally a disease of squirrels, was of particular concern during the Smallpox Eradication Programme in Africa, because the virus could spread to humans, causing a smallpox-like disease. But it also spread occasionally from person to person. Although a rare disease in humans, confined to the tropical rainforest of Central and West Africa, there was the possibility that it could take off as a self-sustaining human infection [11]. Fortunately it is transmitted much less effectively than smallpox. Epidemiologists, who developed a stochastic model, predicted that a maximum of 11 serial transfers were possible in humans, and the longest chain of person to person spread actually observed was through four serial transfers [43]. The virus is known to have infected 400 humans in Central and West Africa; 400 opportunities to produce a mutant strain that spreads well in humans by the respiratory route. So far it has not done so, although presumably the virus has been causing human infection for a long time, unrecognized against the background of smallpox.

Reston virus, a filovirus, like Ebola and Marburg, was inadvertently introduced into a laboratory in Reston, Virginia USA, in 1989, when infected crab-eating macaques were imported from the Phillipines. It seems to have spread among the monkeys by the respiratory route, causing a lethal disease [44], and also infected a few laboratory workers. Fortunately it not only failed to be transmitted from person to person, but also caused no illness in the human host. Again, during the

recent outbreak of a lethal morbillivirus infection in horses in Queensland, Australia [45], two humans were infected and one of them died, with severe pathological changes in the lungs. Here was another opportunity, but fortunately once more the infection was not transferred to other people, presumably because there was little or no growth of the virus in respiratory epithelium. Whatever its natural animal reservoir, this virus will probably again infect horses and perhaps humans, and will have fresh opportunities to generate a variant capable of person to person spread.

Because an Andromeda strain could come to us from biting arthropods or animal reservoirs, the probability is increased as human populations expand and experience more frequent encounters with these arthropods and animal reservoirs. If it arose in a small, isolated community, it would burn itself out and become extinct as soon as all available hosts had been infected, and this may well have happened more than once in the past, but today there are more people, with greater mobility, which means a greater risk of global spread.

AN EXISTING RESPIRATORY PATHOGEN AS A SOURCE?

Could one of the human infections already transmitted by the respiratory route acquire more virulent, even life-threatening properties? There is always the important possibility that a new pandemic strain of influenza virus may arise, causing a frequently fatal primary viral pneumonia. Or the new strain might regularly invade heart muscle. Influenza viruses occasionally invade muscle, for instance influenza B in skeletal muscle in children [46]. New pandemic strains of influenza A are generated by reassortment between bird and mammalian viruses. There are 13 types of haemagglutinin (H) and 9 types of neuraminidase (N), which gives 117 possible combinations and, although 71 of these have been found in birds, for unknown reasons only 3 (H1N1, H2N2, H3N2) have so far appeared in humans. In birds, at least, a single aminoacid change can convert an avirulent into a highly lethal strain [47]. Alternatively, a coronavirus could appear that nearly always caused encephalitis; current coronaviruses only cause common colds and possibly gastroenteritis in humans, but many are neurotropic in their animal hosts [48].

About 10% of bubonic plague victims develop pneumonia, and the infection is then transmissable from person to person by the respiratory route, causing pneumonic plague. The factors determining bacterial localization and growth in the lung and respiratory transmission are not known. Untreated cases suffer almost 100% mortality, but streptomycin and tetracycline are available for treatment and prophylaxis, and there is a moderately effective vaccine.

THE NEED FOR FOCUSED RESEARCH

A catastrophic respiratory infection, the sort that could decimate our species, is always a possibility. Viruses evolve rapidly in relation to their vertebrate hosts, and because of this threat it can be argued that we ought to know more, not only about the mechanisms of virulence, but also about the factors that enable them to be transmitted by the respiratory route. At one time, respiratory transmission was studied intensively, partly for its military interest, and several international meetings on Aerobiology were held in the 1960s [49]. Now we need to know more

about the molecular determinants of respiratory transmission at the cellular and pathogenesis level [21], with *in vitro* and *in vivo* studies of naso-pulmonary capillaries and respiratory epithelium.

We are a vulnerable species, and even in these days of vaccines and antimicrobial drugs infection continues to be a serious threat to our future. If the Andromeda strain ever appears, a basic understanding of its transmission may provide us with the best strategy for defence.

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