VIRUS INFECTIONS OF THE UPPER RESPIRATORY TRACT

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Infection of the upper respiratory tract can be considered the most common ailment of the human race. In the thirties the influenza virus was isolated by Smith Andrewes and Laidlaw by infecting ferrets intranasally with infectious material from patients. They observed also that in convalescent sera antibodies could be shown which neutralised the infectious agent. Three main types of influenza were discovered, the A, B and C type and it was obvious that these viruses were not the only ones which caused respiratory infections. During the last decennium the tissue culture technique was highly improved as a virus laboratory method. The two kinds of tissue culture which are at present the most suited are the use of continuous cell-lines and the primary tissue culture.

The cell lines are more or less stable laboratory strains which can be propagated continually by detaching the cultured cells from the glass wall by trypsin or versene and bringing the cells into a fresh medium. In a few days a new layer of cells has formed on the glass wall, the culture being ready to be inoculated with virus, or can be used again for subculturing.

Primary tissue culture can be prepared from fresh human of animal tissue. A piece of a suitable organ is removed under aseptic conditions and the cells of this organ are dispersed by treatment with trypsin solution. The cells are suspended in a nutrient medium in tissue culture tubes. After a few days a monolayer of cells has formed along the glass wall and the culture can be used for inoculation with virus. These cells are not subcultured. For the isolation of viruses from patients, proper specimens are collected early in the disease. In the laboratory the specimens are treated with antibiotics in order to eliminate bacteria, an inoculated in tissue culture. In most cases the cells show alteraltions — called the cytopathic effect — when the virus propagates in the tissue culture. Identification of the isolated viruses can be performed with the neutralisation test. Specimens of the isolated virus are mixed with specific antisera and these mixtures inoculated into tissue culture. Absence of cytopathic effect indicates neutralisation and discloses the identity of the virus. Although the upper respiratory tract can be considered as an ideal portal of entry for viruses — for example smallpox, measles, mononucleosis — we will discuss only the viruses which induce typical respiratory infections. As these viruses do not confine their attack to particular anatomical sections of the respiratory tract an aetiological classification on this basis cannot be given. It is better to group them into four major syndromes which can be readily observed: atypical pneumonia, influenza, acute respiratory disease and common cold.

The first syndrome, atypical pneumonia, involves the lower respiratory tract and is out of the scope of this meeting. The second syndrome, influenza, can cause symptoms from any part of the respiratory tract and gives usually more or less severe general symptoms. It is a typical epidemic disease. Of more interest to us now are the two other syndromes: acute respiratory disease and common cold.

Acute respiratory disease can be produced by a large number of viruses: adenovirus, respiratory syncytial virus, the reo viruses, parainfluenza viruses, and coe virus. The adenoviruses were originally isolated from recruits with acute respiratory disease, hence the name A.R.D. virus. Afterwards the symptoms adenoiditis, pharyngitis and conjunctivitis were judged so important that the name A.P.C. virus was used, but now these viruses are generally called adenoviruses. They include at least 28 immunological types. Although they mainly produce respiratory infection these viruses often can be isolated from stools, so that we must assume that they are able to give enteric infections. The reo viruses, respiratory-enteric viruses, may be isolated from both faeces and upper respiratory tract. They were originally considered as enteroviruses of the ECHO virus subgroup, but have been removed from this group because of their larger size and other properties differing from those of the enteroviruses. Coe virus is identical to coxsackie A 21 enterovirus.

The parainlfuenzaviruses (4 immunological types) are a subgroup of the myxoviruses, which are named after the fact that they attach to certain mucoproteins. The parainfluenza viruses and the respiratory syncytial virus are real respiratory viruses.

In acute respiratory infection a variety of symptoms can be observed, from rhinitis to acute pneumonitis, from keratoconjunctivitis to bronchiolitis. Usually it is very difficult or even impossible to correlate the symptoms with the causative agent. In order to make a diagnosis of the virus involved either throat and nose washings or tampons have to be sent to the laboratory. Many of these viruses however, are inactivated rapidly outside the body and therefore freezing of the specimens during shipment is necessary.

The viruses can be isolated on primary monkey kidney cell cultures (Reo viruses, respiratory syncytial virus, parainfluenza virus and adenovirus) and on continuous cell lines such as HeLa cells (adenoviruses and Coe virus). The cytopathic effect of some of these viruses shows typical aspects which facilitates diagnosis. The respiratory syncytial virus causes extensive syncytia with intracytoplasmatic inclusions. The adenoviruses are recognisable by the typical nuclair alterations which they produce in the cells.

The parainfluenza group viruses — just like the other myxoviruses: influenza, mumps and Newcastle disease — can be diagnosed easily in tissue culture by the fenomenon of haemadsorbtion. That is, if a suspension of erythrocytes is added to the tissue culture, in which the presence of a myxovirus has to be detected, the erythrocytes are adsorbed to the infected cells. By this reaction an early diagnosis can be made as this haemadsorbtion can be performed before a cytopathic effect is evident.

Although the viruses mentioned above can cause symptoms of rhinitis the majority of common colds is caused by rhinoviruses. They resemble the enteroviruses in their size and density but differ from them in some physical properties. The rhinoviruses are relatively difficult to culture and in this respect they can be divided in two main biological groups the H type and the M type.

The H type grows in human cells only, especially in human embryo kidney cells or human embryo lung fibroblasts. These cells are not readily available

for all laboratories and this can be a disadvantage for the diagnosis. The M type viruses multiply in monkey kidney cells as well in human cells. As real viruses of the nose they prefer an incubation temperature somewhat lower than body temperature, i.e. 33°C. Both the H and the M strains have several serological types. All strains produce colds when administered to volunteers with low antibody levels. High levels of antibody are protective against infection. Laboratory animals are not susceptible to experimental infection. The type of illness produced in volunteers may vary a little depending on the strain used. Rhinoviruses can be isolated in tissue culture from 20—30 % of common cold cases, but it must be remarked that there are still strains of these viruses which can not grow in tissue culture but can be detected by inoculation into volunteers.

Because of difficulties in the isolation of respiratory viruses — mainly due to rapid inactivation during shipment to the laboratory or in the case of rhinoviruses the lack of suitable tissue — in many cases a serological diagnosis is preferred. The disadvantage that this serological diagnosis takes at least 14 days is of no importance as a rapid diagnosis is not needed for therapeutic measures.

As respiratory infections are widely distributed no conclusions can be made from the titers, high or low, determined in a single serum sample. At least a fourfold increase of the titer in two serum samples — one collected in the acute stage, the second 2 to 3 weeks later can be considered as evidence of infection. A complement fixation test, with antigens prepared in tissue culture or incubated eggs, can be performed in practically all cases. An advantage is that this reaction is easely performed and gives rapidly increasing titers. But many viruses are so closely related that no conclusion can be made in respect to the type involved. The adenoviruses have all a common complement fixing antigen, the parainfluenza viruses show considerable overlapping. For determination of the type of the virus involved a neutralisation test has to be performed. In the myxoviruses — which show the fenomenon of haemagglutination — a type specific haemagglutination inhibition reaction can be performed. Vaccination against respiratory viruses, with the exception of influenza, is still in an experimental stage. The large number of viruses and the many different antigenic types gives us little hope of complete prevention of respiratory infection by active immunization of the population.

SUMMARY

Upper respiratory infection is a common ailment in man. The adaptation of tissue culture technique to the virus laboratory has resulted in the isolation of a great number of viruses which produce infections of the respiratory tract. None of them confine their activity to a specific anatomical site, so a classification in this respect is impossible. This implies also that diagnosis of a viral disease has to be made in the laboratory. Specimens for virological diagnosis have to be sent in a frozen state to the laboratory as otherwise the virus will be inactivated during shipment. The viruses can be grown in tissue culture.

A serological diagnosis is more easily made. Two serum samples taken at a suitable interval are needed in order to provide evidence of infection by an increasing atibody titer. The neutralisation test discloses the type of virus involved, the complement fixation test mostly indicates the group only.

INFECTIONS VIRALES DES VOIES RESPIRATOIRES SUPÉRIEURES

L'emploi et l'adaptation de cultures cellulaires en tant que technique de laboratoire virologique a résulté dans l'isolation d'un grand nombre de virus provoquant des infections respiratoires. Le fait qu'aucun de ces virus ne limite son activité à une certaine région anatomique, rend une classification impossible à ce point de vue. Les virus ont un vaste spectre clinique et on peut distinguer quatre grands syndromes:

— pneumonie à virus atypique,
— influenza (grippe),
— infections respiratoires aiguës — provoquées par les adénovirus, les reovirus, le coevirus (Coxsackie A 21), le virus respiratoire syncytial et les myxovirus parainfluenzae.
— rhume provoqué par les rhinovirus.

Il faut que le diagnostic d'une maladie virale soit fait au laboratoire. Pour cela des écouvillons du pharynx et du nez doivent être envoyés au laboratoire en état de congélation afin de conserver l'activité du virus pendant le transport. Au laboratoire les virus sont cultivés sur milieux cellulaires — cellules rénales de single, cellules HeLa ou cellules KB — où ils provoquent des dégénérations : l'effet cytopatholgène.

Un diagnostic sérologique est plus facile à faire. Les anticorps contre les virus respiratoires sont très répandus dans la population. Il est évident que le diagnostic des affections virales ne peut être fait sur un seul sérum. On a besoin de deux échantillons de sérum, pris dans la phase aiguë et pendant la reconvalescence afin de prouver une augmentation du taux d'anticorps dans les réactions de fixation du complément et les réactions de neutralisation.

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