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Mini-Review

A view from the Common Cold Unit

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Summary

I have been asked to stand back and describe in broad terms the view I have had of common colds – probably the most frequent of acute human diseases and a long-lasting scientific problem – and in particular our recent work on antivirals. I should be able to do this for two reasons. Like everyone else I have suffered from colds, but in addition I have been studying the problem from the virological and clinical point of view for over 35 years – for the last 31 at the Common Cold Unit, Salisbury. As a result I may have problems with perspective – it is not possible to give a personal view and at the same time to describe something from every possible angle, and quite impossible to be comprehensive, but I have done my best and readers will make their own judgements and corrections.

Common cold; Rhinovirus; Clinical trial

Introduction

Soon after starting research I learnt for myself how difficult it might be to detect a 'new' cold virus. In the early 1950s in the laboratories of the Rockefeller Institute in New York, I had just found out how to make roller tube tissue cultures which would support the growth of influenza viruses. I took some of these cultures and inoculated washings from a patient with a cold. After a few days a cytopathic effect appeared and I was all excitement, only to discover shortly afterwards that the cultures had a low grade infection with a cytotoxic bacterium. I discarded the experiment and got on with other work. In due course I returned to the UK, was appointed to the staff of the Medical Research Council (MRC) and worked in Sheffield on respiratory viruses, such as adenoviruses and influenza; but circumstances led me to join the work on

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poliovirus vaccines and on the role of recently cultivated enteroviruses, all of which seemed to take me further and further from respiratory viruses. But I was then asked to work at the Common Cold Unit, Salisbury. In one way this meant that my outlook had to be much narrower, but in another it broadened greatly the possible approaches. I could now prepare pools of filtered nasal washings from individuals infected with clinical material, and thus had a documented stock of viruses which could not be propagated in the laboratory. We could also use the volunteers as a specific and sensitive test for the presence of a common cold virus. Thus we systematically tested for the growth of viruses in tissue cultures by inoculating culture medium as nasal drops into groups of volunteers living in isolation and observing whether they developed colds or not.

But how did it come to pass that there was a Common Cold Unit at all, and was it really a good idea to have one? Colds and minor respiratory catarrhal illnesses must have been occurring for centuries, and are mentioned in diaries and literature. Late in the 19th century, with the rise of bacteriology, colds were actively investigated with the newly developed methods. Indeed by the 1930s it took a volume of 710 pages to summarize the published investigations but the virus theory was dismissed in only nine of them (Thomson and Thomson, 1932). Some of the results reported seem bizarre these days but it is possible to guess at how some of them were obtained. For instance it seems that some workers did not distinguish clearly between the early acute illness and subsequent catarrh, sinusitis and secondary bacterial infections. Of course, good methods were available to culture and characterize common organisms such as pneumococci, haemophilus species and streptococcus haemolyticus, but there was much confusion with other organisms such as *Neisseria* and, as we would now call them, *Branhamella*. Anaerobic bacteriology was also beginning and gram-negative filter-passing anaerobes were alleged to exist. As a result a wealth of possible causative organisms were cultured. Even when it was realised that some bacteria might be harmless symbionts, it was possible to convince oneself that certain organisms appeared in the pharynx more frequently in colds than in good health. One study tackled this problem with really careful bacterial assessment, and a careful and impartial experimental design for collecting clinical records and bacterial specimens (Mills et al., 1928). This concluded that although these anaerobic bacteria came and went in the airways they were a heterogeneous group and their presence was not related in time or quantity to the occurrence of cold symptoms. It is easy to look back at those studies with the confidence engendered by a view down the 'retrospectoscope' and see them clearly as a decisive observation. However, in the context of the time one can imagine how much authors might be influenced by confusion over whether filtrates transmitted colds and if they did what it meant. Powerful evidence was also being collected at the same time to show that haemolytic streptococci were associated with pharyngitis and many of its complications and that certain serotypes of pneumococci were unquestionably linked to pneumonia. After all it is less than ten years since we lived through a similar but

brief period of confusion, claims and counter-claims until the HIV virus was cultured and its relationship to AIDS was documented.

In fact specific evidence that viruses caused colds had been obtained long before the 1930s by the appropriate methods of the time. Kruse published in 1914 in the Munich Medical Weekly the results of an experiment in which he passed nasal washings through a bacteria-tight filter, inoculated it into volunteers and observed colds. This result was overlooked, possibly in part because of the outbreak of war, but it was also discredited because his volunteers were not isolated and so a few who were given uninfected material nevertheless developed colds; there was also the question of whether filtration through Seitz filters or Berkefield candles would remove all bacteria. These needed to be carefully checked to ensure that they *did* hold back all bacteria and this could be done as a control test. Indeed it may be because these tests were not properly done or interpreted, that it was believed by many that anaerobic organisms could exist in a filterable form.

In my view the best attempt to resolve this confusing situation was made by Dochez's group at the Rockefeller Institute (Dochez et al., 1930) where they very carefully filtered nasal secretions and checked that they were bacteria-free and showed that they produced colds when given to chimpanzees or human beings held in strict isolation (which was shown to prevent colds in uninoculated subjects). However, further problems lay ahead. Having shown that colds really could be due to viruses the same group then set out to culture the virus using the technology of the day, namely cultures of chopped chick embryo tissue, held under a layer of paraffin (Dochez et al., 1931): they believed they could produce colds with such material. C.H. Andrewes (personal communication) tried to repeat this experiment in England, and even brought cultures from New York by ship, but failed to get convincing data, and concluded that the results were due to irritation produced by autolysed tissue. However, the studies on the viral nature of colds were well designed and executed and although they did not immediately convince all those in the field they stood the tests of time and repetition and caused the edifice of studies of bacterial 'causes' of colds to be weakened and eventually to crumble.

The Common Cold Unit and 'the' common cold virus

It would be an interesting piece of social and historical research to define the thoughts and beliefs and the social background which led to the founding of the Common Cold Unit at Salisbury, UK in 1946. We know that there were unanswered scientific questions and that C.H. Andrewes, already known for passing human influenza virus to ferrets (Smith et al., 1933), was anxious to do more experiments on colds using human volunteers in a similar way. Furthermore the old American Red Cross/Harvard Hospital site had lain vacant since the US Army had left it empty when they moved their pathology laboratory to Paris. But there were very few virologists in the UK, the country

had desperate economic problems in the rebuilding of millions of damaged homes, repairing and re-equipping factories and generally developing an effective peacetime economy. Yet at the same time there was a general hope for a better country and a better life in the post-war years. Perhaps it was also believed that the task was simpler than it really was: that some months or years of research would reveal 'the' common cold virus against which a vaccine would be made (Andrewes, 1949). War-time experience had also given rise to the belief that so-called 'minor' infections could significantly reduce production, and it was perceived that this would be important in peacetime too. Whatever the truth may have been, a joint plan was formulated in early 1946 – the Ministry of Health would support the accommodation of the volunteers, up to 20 or 30 each two weeks, the Medical Research Council would fund the laboratory work and staff, and the Ministry of Works would look after the buildings and the basic services. In a matter of a few months the buildings were converted, a small staff was assembled and in July of the same year the first group of seven student volunteers arrived to be the subjects for the first of a series of trials which continued without a break for 43 years and involved almost 20 000 visits to the Unit, and over 18 000 individuals.

One then has to ask certain questions. Was it really a good idea? Was it worth it? Would research have gone on equally well without it? Such questions are in many ways unanswerable. It is always important to clear away unsound research reports when starting in a new field, and we may at this distance not value adequately the early work done, without much publicity, to show that the cold virus could *not* be induced by chilling, and could *not* be transmitted to animals such as cats, or passaged successfully in embryonated eggs as had been claimed by previous workers (Andrewes, 1951).

Then there were the first, and very careful, experiments to show the passage of a virus in cultures of human embryo lung, as used by Enders and colleagues in their first experiments on polioviruses (Andrewes et al., 1953). The virus grown is now known to be a rhinovirus type 9 but was then called 'the' common cold virus. Of course rhinovirus 1a was grown away from the Unit by applying to respiratory specimens other techniques developed from those of Enders, i.e. roller tube cultures of monkey kidney cell cultures and observation of a cytopathic effect (Price, 1956; Pelon et al., 1957).

However it is my guess that the development of general methods using tissue cultures, and later organ cultures, of cells, was generally faster and more logical at the Unit where we were able to combine the use of new culture techniques with the inoculation of volunteers to indicate that a cold-producing agent was growing to even a limited extent. We first found a method for propagating many different 'cold' (actually rhino-)-viruses in roller tube cultures of human embryo kidney cells at 33°C for two passages, without a cytopathic effect (CPE) (Tyrrell et al., 1960). The medium was modified, first to allow serial passage and the development of interference, and then to allow a rapid CPE to occur (Tyrrell and Parsons, 1960; Hitchcock and Tyrrell, 1960). Others later found more convenient susceptible cells. Once fully developed, these methods

could be used in any laboratory, the biology and drug sensitivity of viruses could be studied anywhere and their presence in clinical material and their relationship to disease either in volunteers or in the community could be determined.

Furthermore, the old empirical idea of the existence of 'the common cold virus' was soon replaced by data collected by epidemiologically controlled field studies in which infection was detected with a range of viruses following which their pathogenicity was confirmed and studied by inoculating them into volunteers and observing their effects. These studies proved that typical colds could be produced by a wide range of viruses including some enteroviruses and paramyxoviruses, but certain viruses, namely rhinoviruses and coronaviruses were the main causes of colds and thus could be used as laboratory reagents in the search for specific antiviral treatments.

Thus, after years of frustration the work of the Unit developed to the point where relevant human infections could be produced at will and precisely documented by clinical and laboratory methods – this was the ideal setting for preliminary trials of the efficacy of antiviral drugs or vaccines without which nowadays it would rarely be justifiable to go on to a field study of naturally acquired colds.

Searching for prevention or treatment

As time went on the Unit also played a role in providing a continuing focus for work on subjects, such as the spread and pathogenesis of colds and antiviral treatment, which went in and out of fashion. For instance early experiments on the use of interferon for the prophylaxis of colds had to face various problems. Once we had even small amounts of human interferon (Sutton and Tyrrell, 1961) we could show in vitro that it would render human cells resistant to a wide range of viruses capable of causing colds, but there was a general view that such treatment would never protect the respiratory epithelial cells, defended as they are by the mucociliary apparatus. Furthermore the first interferon preparations were of very low potency. Later chemically synthesized antivirals were also disappointing, but interferon experiments continued with purer and more potent preparations until 1973 when the idea of antivirals for the treatment of colds finally came of age and rhinovirus-induced colds were prevented at the Unit by spraying human leucocyte interferon intranasally (Merigan et al., 1973). This result was confirmed and shown to be due to interferon itself when colds were prevented with leucocyte interferon which had been purified by means of monoclonal antibody (Scott et al., 1982a) or synthesized in *E. coli* by recombinant DNA technology (Scott et al., 1982b). This work has been expanded by other groups and early trials are reviewed by Scott and Tyrrell (1985).

The continued existence of at least a few test sites in the world encouraged research to continue on antivirals until eventually a chemically synthesized molecule was detected which also had a clear-cut beneficial effect against colds (Al-Nakib et al., 1989). After further development such drugs will no doubt be studied in the community as interferon has been.

There is a common view that the time for such work has passed, that such specialized expertise and resources are not needed, that high technology is all that is required to provide further advances. It is of course important to ensure that research on colds, or any other infection, exploits the contributions of the best current laboratories and their equipment – electron microscopy in the '60s and molecular biology in the '80s. Such methods greatly increase the power of the science but are not able to answer crucial questions on whether new molecules provide benefit to humans, and how they can be applied. In the absence of an animal model and in the face of the multiplicity of causative viruses, it is important to have a 'half-way house' between the test tube and studies of natural colds to identify promising drugs and find out how to use them clinically (Beare and Reed, 1977). Important elements of this are the willingness of the public to help, a place where they like to come for the experiments and the invisible relationships built up with the experimental team. It will be essential to reproduce these if successful experiments of this sort are to be done in the future.

Advances in the detection of viruses and antibodies have also been used to study other aspects of respiratory infections in man. It was possible to use the volunteer resources to study and measure the pathogenicity and immunogenicity for man of influenza viruses modified in various ways, for example, by serial passage in eggs or mice and reassortment with epidemic strains, with a view to developing live attenuated vaccines; such work complemented that done in the USA and USSR and has continued up to recent years and now includes the detailed molecular analysis of the candidate viruses (Beare, 1975; Nicholson et al., 1987; Oxford et al., 1990).

It was also possible to document the way viruses damage cilia and slow mucociliary transport (Wilson et al., 1989) to look for the role of atopy and possible mediators in producing respiratory symptoms (Callow et al., 1988) and to document local physiological parameters such as nasal blood flow (Bende et al., 1989). Psychological factors, such as personality and 'stress' were also found to be important. It was possible to use volunteers with colds as a mild but definable 'organic' disease in which one could document quite rigorously (Totman et al., 1980; Cohen et al., 1991) effects which very probably occur in other less common but more serious diseases, but which could not be studied experimentally for ethical and practical reasons. Other experiments showed that human performance is impaired by colds and influenza (e.g. Smith et al., 1988a) and a start was made in looking for the role of virus-induced humoral factors such as interferon as mediators of these changes (Smith et al., 1988b). These findings were not intended to lead to new treatments directly but complemented work done elsewhere to provide a fuller understanding of the disease, which in turn could open up new forms of treatment. They also provided a number of new techniques, such as neuropsychological performance tests, for the assessment of the systemic effects of the infection and for documenting the benefits of treatment.

The international scene

The Common Cold Unit was not the only group to be studying respiratory viruses in human volunteers. A young man, George G. Jackson, met Dr. Andrewes and visited the Salisbury Unit. He saw the possibility of using the climatic chamber at the University of Chicago to study the possible effects of chilling on colds (Dowling et al., 1958). With Dr. H.F. Dowling and others he published the results of studies on many hundreds of volunteers (Dowling et al., 1957). Unfortunately they were unable to isolate their volunteers and thus some of their work on the identity of the viruses they studied was not entirely clear, though they did pioneering work on the factors which influence susceptibility, on the effects of amantadine and on other topics.

A great deal of excellent laboratory work was done at the NIH Bethesda MD, detecting new viruses in respiratory disease of childhood, for instance the para-influenza viruses by Chanock and his colleagues (Chanock et al., 1958). Although it was very likely from the circumstances that they caused the diseases affecting the patients in which they were found, it was desirable to try and fulfil 'Koch's postulates' and show that the cultured virus would cause respiratory disease in man. Thus they were led to inoculate adults in room isolation and showed that they were infected and developed a disease which resembled a common cold, rather than the more severe lower respiratory disease which they found in children (Reichelderfer et al., 1958). The group also worked on rhinovirus colds, and used volunteers to do important early studies on the role of antibody in immunity to infection (Cate et al., 1964). Another group at the NIH was able to exploit the technology developed as a result of concern with biological warfare. They generated accurately defined aerosols of various respiratory viruses and developed equipment to enable volunteers to inhale known amounts of these (see Knight, 1964). Thus they could calculate the amount of virus delivered as an aerosol which was required to initiate an infection: furthermore they showed that when given as a fine aerosol, viruses which otherwise invade the upper airways and cause colds would invade the lower airways and cause lesions in the lungs and bronchi. When some of the group transferred to Baylor University in Texas they performed volunteer experiments in local prisons, for instance on live influenza vaccines.

A continuing centre of volunteer work was set up in the University of Maryland, Baltimore. This group also worked with known viruses, and on a number of occasions performed studies on influenza viruses and candidate antiviral drugs (e.g. Togo, 1972).

In more recent years an outstanding centre was set up in the University of Virginia, Charlottesville under J. Gwaltney and F.G. Hayden. The group had a particular interest in rhinoviruses and studied them in the field. They also set up arrangements for using, at intervals, local resources, such as a motel, in which to conduct studies in which volunteers were rigorously isolated and studied clinically and virologically. They also had a high reputation for their work on antiviral treatment, and studied the effects of intranasal interferon etc. (see below). They showed by volunteer studies that it was possible to transmit

rhinoviruses by contamination of fingers. However, the importance of this is still not decided (see below).

On an occasional basis, E.C. Dick at the University of Wisconsin also set up volunteer experiments on the transmission of colds, some of which are mentioned later.

All this work reached a peak in the 60's and 70's and had been significantly reduced by the late 80's. Nevertheless it was of great value that work went on in different centres. For one thing the numbers of volunteers in any one centre tended to be small. Though power calculations were not done in those days, we used, at the best, scores of subjects. Effects were sometimes modest and although they were apparently significant most of us felt much more comfortable about our conclusions when experiments had been repeated elsewhere with similar results. It was in fact remarkable how closely the results agreed at times, for instance on the clinical effects of para-influenza viruses or the protective effect of interferons. On other occasions hopeful results of trials with candidate drugs could not be repeated and so work moved on to others. Finally the existence of these groups created a sort of loosely-knit club. Key reagents were exchanged, results were discussed formally or informally on site, we learned from each other and many personal friendships were established.

Ethical considerations

The reader will realise that the Common Cold Unit was set up at a time when the Nuremberg Trials and the Helsinki declaration were in the mind of the public and of experimental scientists. The Medical Research Council enunciated its own statement on studies in human beings some time later. Nevertheless, no formal review bodies existed for much of the Unit's life. However the type of experiment to be done was carefully considered by the Head Office. In addition when it was suggested that good conduct prisoners or servicemen might be recruited, it was decided that being under strong discipline they could not be considered as able to volunteer freely – though when they were on leave servicemen could come on the same basis as civilians if they wished, few did.

All the volunteers made the first approach to the Unit, in response to media reports or personal recommendations, so there could be no undue pressure at that stage. They were sent a fairly full description of the Unit and the purpose and nature of the experiments, including the need to restrict their movements and contacts, and this was repeated and amplified if they finally came to the Unit – though many would-be volunteers never followed up their initial contacts. It was always true that volunteers might be dropped from trials. Some, for instance those who developed a quarantine cold, might stay on for their 'holiday' or leave early, but others, a small number, just left because they did not like the place.

It was Unit policy not to use invasive procedures, and there were even long and serious discussions before it was finally decided to collect blood samples, at least for some studies. Furthermore, it was the policy not to administer any

virus which might produce serious, let alone possibly lethal disease – thus although unmodified 'common cold' viruses were given, influenza viruses were always passaged in order to attenuate them. Thus the general principles that all volunteers should give their 'true' or 'free' and informed consent and that only non-hazardous experiments were acceptable were being applied before they were encoded in ethical committee rules and procedures.

In spite of this careful approach some of the experiments done then would not be acceptable today. Extensive use was made of human embryo tissue – this was obtained from patients with no clinical history or evidence of infection and was handled in a 'clean' area. However it was tested only for bacterial and fungal infection before administration to volunteers. Furthermore on some occasions virus propagated in monkey kidney cells was used, and although the cultures were tested for haemadsorbing and cytopathic agents, we did not know about non-cytopathic organisms such as SV40 and so did not test for them.

When local ethical committees were introduced we were able to comply with their requirements with no more difficulty than that occasioned by the extra paperwork. I remember that at the height of the controversy about 'human guinea pigs', I was in a public debate pitted against a very able and determined lady who took the line that all experiments on human beings are unethical and unsafe. At the end of the hour she summarized her views 'probably what *you* do Dr Tyrrell is alright, but I still think .....'. Looking back one can see that our precautions were incomplete, due to lack of knowledge at the time, but the guidelines we were following in the conduct of the studies were sound and very close to those enunciated in a recent report of the Royal College of Physicians (1986).

Possible strategies for prevention and treatment

Common colds, like any other infectious disease, might be brought under control by intervening in a number of ways. These are to prevent transmission, enhance host immunity, limit virus replication, or prevent the unwanted effects on the hosts, i.e. symptoms and signs in the nose and elsewhere. All these have been tried in one way or another and in the following brief comments I summarize my current views on each and try to describe where I think they may lead theoretically or practically.

(1) Transmission

In the early days the mode of transmission was only guessed at and my text book of medicine said that colds were transmitted by talking, sneezing or coughing. Work in the Unit and elsewhere suggested that colds had been transmitted from children to volunteers by the airborne route (Lovelock et al., 1952) but no techniques were available to detect virus in the air or to quantitate virus in the mixture of droplets ejected into the air by a sneeze. However, the nose collects particles from air during inspiration at the rate of about 10 litres per minute, but nevertheless transmission of infection is relatively infrequent

(e.g. Hendley et al., 1969). If each infection is initiated by the inhalation of one infectious unit, it is clear that a low concentration of such units in the air would be sufficient to explain the epidemiological findings. In volunteer studies with coxsackievirus A21 it was shown that colds could be transmitted from one end of a long room to the other when only air contact was possible (Couch et al., 1970). We demonstrated how Coxsackie A21 virus, which is very like a rhinovirus, is shed as drops and droplets into the air by a subject with a cold and that similar droplets infect the nose efficiently when inhaled (Buckland et al., 1965). However, there was very little virus in saliva and so little became airborne when talking.

In 1973 Hendley and colleagues at the University of Virginia reported that virus could get onto the skin or fomites of patients with colds (Hendley et al., 1973). We knew that virus could infect if placed onto the nasal mucosa by a contaminated swab or finger (Bynoe et al., 1961; Buckland et al., 1965). Thus transmission by indirect contact would be possible. However, the really difficult question is how often this happens or rather what is the relative importance of transmission by the contact and the airborne route, and quantitative studies seemed to us to show that the finger route was relatively inefficient (Reed, 1975).

In recent careful experimental work, manual transmission in a group playing poker was prevented by using splints or large plastic collars; yet rhinovirus infection was freely transmitted, so the airborne route must have been of major importance in those circumstances (Dick et al., 1987). Nevertheless in an earlier experiment they showed that viricidal tissues prevented transmission during an experimental poker game (Dick et al., 1986). However, in order to test whether finger transmission was important in families, Gwaltney's group set up a placebo-controlled household study to block the transmission of rhinovirus colds through the fingers. This turned out to be a difficult experiment to conduct and although there was a trend in favour of the treated group there was no large or statistically significant overall effect (Farr et al., 1987). Early work on the use of UV light in school classrooms showed that the transmission of measles could be slowed down by impeding airborne transmission, but neither this nor the use of viricidal tissues seem practical methods of preventing a substantial amount of transmission. Water treatment and food hygiene have had a profound effect on water and food-borne diseases. It seems unlikely that anything as successful can be developed for respiratory virus infections but, nevertheless, good ventilation and hand hygiene should be encouraged and are likely to do some good.

(2) Host resistance

Soon after the first rhinoviruses were cultured, a collaboration was set up to produce an inactivated vaccine against RV2. This was given intramuscularly to volunteers before they came to the Unit and they proved to be resistant to colds produced by intranasal challenge with live virus of the same serotype (Scientific

Committee, 1965). Although others believed secretory antibody to be of particular importance, we have done recent studies which show that circulating antibody (which was stimulated by the vaccine) is long-lasting and protects significantly against illness, although secretory antibody is effective in preventing infection (Rossen et al., 1966; Barclay et al., 1989). Now that the sequence and structure of rhinoviruses is becoming better known, it is possible to identify possible linear peptide epitopes on the virus surface. This in turn suggests the possibility of searching for peptides which could be given as a killed vaccine to produce neutralising antibody (Francis et al., 1987). Of course, there are many difficulties along the way, such as antigenicity, the need for T-cell epitopes, the variability of the human immune responsiveness etc., but the possibility of vaccination against rhinoviruses is worth exploring further. Indeed we have entered a new era in which the antigenic sites of two representative rhinoviruses types, 14 and 1a, have been mapped onto 3-dimensional models of the virus particles (Rossman et al., 1985; Rossman, 1989; Appleyard et al., 1990). They turn out to be located on the edges of the 'canyon' around the 5-fold symmetry axis of the particle. We have determined that human volunteers make antibodies against at least 3 such sites on rhinovirus type 2 identified by mouse monoclonal antibodies. Challenging the volunteers shows that the presence of such antibodies is associated with resistance to virus infection (Carey et al., 1992). Many sites seem to be conformational, and a specific amino acid may be involved in the sites seen by more than one monoclonal antibody, so the problem is undoubtedly complex and more basic work is needed before sites on the edge of the 'canyon' can be fully identified and mimicked synthetically for one serotype, let alone for the 100-odd types now known. It has however already been suggested that it may be possible to generate neutralising antibody against the conserved sequences deeper in the 'canyon' which is the suspected site of interaction between the virus and the cell receptor (Rossman, 1989).

There has been more success in applying the discovery of interferons. The alpha and beta interferons (IFNα and -β) are produced by virus-infected cells and protect cells with which they come in contact by triggering the development of a state of resistance to virus infection. It is now well known that exogenous interferon, delivered as a nasal spray, protects the human from both experimental and naturally acquired common colds (see Scott and Tyrrell, 1985 which includes references to all other groups, in particular those at Charlottesville). However, in family studies it was effective only against rhinoviruses, and prolonged administration in doses big enough to protect gave rise to nasal symptoms such as mild nose bleeding, stuffiness, etc. Thus interferon cannot be used for continuous prophylaxis. This was found with IFNα2a, but there was some hope that IFNβ might show a different ratio between the concentrations giving protective and adverse effects, but this has not proved to be so. IFN gamma (IFNγ) has also been tried at doses with equivalent antiviral effects in vitro. However, it had no beneficial prophylactic effects, indeed it seemed to make symptoms worse (Higgins et al., 1988),

possibly because it enhanced the production of virus receptors (ICAM-1) on the cell surface (see below). Nevertheless there are many more interferons and others can be generated by gene manipulation or synthesis. Some are being studied in the laboratory, and perhaps one will be found that is relatively less 'toxic' and so could be used for prophylaxis. One of the problems, however, is that there is no satisfactory laboratory test for the local inflammatory effect which is the 'toxic' effect that limits clinical use.

Meantime attempts are being made to devise schedules by means of which the antiviral effect would be retained but the local adverse effect would be lost. However, it seems that once the dose has been reduced to the point when it produces no nasal symptoms it also has no detectable antiviral effect (Monto et al., 1989).

Prophylaxis could be useful in clinical practice under certain conditions, but it would be much better to be able to improve the course of colds by drugs administered after symptoms began, i.e. therapeutically. When natural and recombinant interferons were tested in this way against experimental influenza and rhinovirus infections in volunteers, they were no longer effective, possibly because by then the subject was already producing substantial amounts. Recently we have tried again with colds induced by RSV (Higgins et al., 1990) because it had been reported that RSV infections in children did not induce endogenous interferon, so that in this case added interferon might be expected to have an effect: unfortunately although IFNα given prophylactically was highly effective, when given in maximum practicable doses immediately after symptoms appeared it conferred no benefit.

A third approach to enhancing the host's immunity would be to use locally administered immunomodulators. These might be effective irrespective of the biological or serological type of virus, and might be free of the local adverse effects of interferons. We have recently investigated two of these. One was a muramyl dipeptide derivative which protected mice and guinea pigs when given in small doses intranasally prior to large doses of influenza viruses. However, when given in the maximum acceptable dose to volunteers it did not improve the clinical or laboratory parameters of experimental influenza, even though the strain used was partly attenuated and the dose given was not large (Higgins et al., 1989). A thioguanosine derivative has been found which protects mice against coronavirus infection by enhancing their resistance (rather than by a direct antiviral effect) and this has also been studied in volunteers using colds induced by a coronavirus; analysis indicates that either it was given in too low a dose, or that it does not enhance the primate response in the same way as that of the rodent (Higgins et al., 1991). In this field we are at a similar stage to that of work on interferons between 1957 and 1973 – we know that there are attractive theoretical possibilities but we do not know whether any such method can be made to work in man or what the 'benchmarks' for the properties of a candidate drug should be.

(3) Find new families of antirhinovirus molecules

As an expression of our belief in the possibility of antiviral treatment for colds, we tested a variety of antirhinovirus molecules derived from independent screening programmes, for example by the Wellcome Foundation (dichloroflavan) and Nippon Roche (an antiviral chalcone). It turned out that these all inactivated virus infectivity by binding to particles, and stabilized the particles against the effects of low pH and heat. The drugs were very potent when tested in vitro and when dichloroflavan was dissolved in oil and when a prodrug to the chalcone was developed they were well absorbed when given by mouth. Unfortunately they were not active in volunteers challenged with a sensitive rhinovirus, apparently because they did not enter nasal secretions, but they were still inactive when formulated as intranasal sprays (Al-Nakib et al., 1987a and b). Furthermore a drug from Rhone-Poulenc with a related mode of action but more water-soluble had no effect either.

While all this was going on there was a substantial programme of research on antipicornavirus drugs by the Sterling Winthrop group. This started with the discovery of the effect of arildone on enterovirus infectivity and led to the discovery of disoxaril and its many congeners. It was particularly important that Smith et al. (1986) studied crystals of rhinovirus soaked in disoxaril and showed that it bound to the virus capsid in a 'pocket' at the base of the 'canyon' round the 5-fold axis of virus symmetry – this region is probably involved with virus entry and uncoating.

The Janssen Foundation was also looking for anti-rhinovirus drugs and evaluated many molecules, one of which was developed as a possible drug (R61837): 3-methoxy-6-[-4-(3-methylphenyl)-1-piperazinyl] pyrazidine (Andries et al., 1988). This drug also bound to susceptible rhinoviruses and inactivated and stabilized them, and studies on drug-resistant mutants showed extensive cross-resistance, including dichloroflavan and disoxaril. This indicated that the site of action for this and the above-mentioned drugs was near to that of the Sterling Winthrop molecules (Dearden et al., 1989). R61837 proved effective against rhinovirus type 9 in volunteers when given prophylactically as an intranasal spray and a series of 3 trials suggested a number of interesting conclusions (Barrow et al., 1990b).

(1) Unlike interferon, it appeared that pretreatment was not important, but that if one discontinued treatment too early symptoms returned.
(2) Treatment which began after infection but before symptoms appeared was effective, but treatment which began after symptoms appeared had no beneficial effect.
(3) There were hints that volunteers given treatment until the colds in the controls had recovered (i.e. for six days) might subsequently develop colds after treatment was withdrawn, i.e. the drug was suppressive rather than curative.
(4) In studies on volunteers given short courses of treatment virus shedding was apparently prolonged and drug resistance viruses could be recovered,

particularly after the drug was withdrawn (Dearden et al., 1989).

Thus this type of antiviral treatment has quite different characteristics from those of say, intranasal interferon or of acyclovir against herpes virus, and much more research is needed. For one thing the molecule has very different activities against different serotypes of virus (we tested it against RV9 in volunteers because it had maximum activity in vitro against that serotype). For another thing more treatment regimes must be tried, in order to avoid return of symptoms and the appearance of drug-resistant viruses. Molecules with different antiviral spectra should be synthesized and investigated; they might be used in combination in order to produce a preparation which is active against a full range of serotypes, or it might be possible to exploit the synergism between different molecules which has been detected in vitro.

In this area we can see real possibilities of 'designing' drugs or drug combinations (Ahmad and Tyrrell, 1986). It is now known that there is a relationship between the serotype of a rhinovirus and the molecular structure of the drugs which inactivate it. Indeed one can think of these molecules as probes for the hydrophobic interior of the virus particle, just as monoclonal antibodies are probes for its surface. The group of M. Rossmann at Purdue University have now defined the site at which R61837 binds to the virus capsid and find it to be very similar to the site used by disoxaril: this may make it possible to improve chemical structures by reasoned choice rather than by trial and error (Chapman et al., 1991).

(4) Blockade of virus receptors

Rhinoviruses are very variable antigenically and it appears that they also vary considerably in sensitivity to the most potent antiviral drugs. However, it has been known that they fall into only two groups as judged by their ability to infect cells in tissue cultures, namely those that can infect only human cells (H strains, or major receptor group) or those that can infect both monkey and human cells (M strains or minor receptor group).

Several groups have been trying to identify the receptor molecules that are involved in virus entry and are responsible for this specificity. A receptor molecule for the major group has now been identified and purified (Tomassini and Colonno, 1976) and recently has been cloned and sequenced by three groups and identified as ICAM-1 (intercellular adhesion molecule-1) (e.g. Staunton et al., 1989) which was previously known as a surface component of many cells including endothelium, is involved in interactions with lymphocytes, and is expressed in response to various cytokines including IFNγ. Antibodies against ICAM-1 inhibit virus growth. Thus antibodies or other molecules that bind to virus or soluble ICAM-1 might be used to prevent and treat infections with major receptor group rhinoviruses. There will no doubt be vigorous research to find useful products of this type and also corresponding blockers for minor receptor group infections.

(5) Blockers of mediators

It is common practice to refer to widely used folk – or OTC – remedies for colds as 'symptomatic' and to mean this pejoratively. However, this seems to me to be unfair. It would be ideal to treat the infection by attacking the infectious agent, but it would be practical to commence this treatment only after symptoms and signs have developed. Such treatment might prevent the virus leading to more widespread disease, but this is uncommon and nasal symptoms will only subside gradually even if the infection is 'stopped in its tracks'. It is therefore arguable that in the case of infections like coronavirus or rhinovirus colds, which are normally quickly self-limited, the best approach would be to relieve the patient's discomfort and disability and leave their immune system to take care of the virus. Furthermore in the case of viral and bacterial infections of the gastrointestinal tract, most of those that lead to diarrhoea are best handled by managing their physiological effects, usually by oral rehydration, while the immune system controls the infection.

The trouble is that we know relatively little about the way in which the nose responds to these infections, and therefore use rough measures to control the symptoms, e.g. administering a general adrenergic stimulator to reverse the vasodilation in the nasal mucosa. We know, however, that antihistamines prevent specifically the symptoms of hay fever and allergic rhinitis because histamine is a key mediator in the pathogenic chain of these diseases. We do not have equally precise information for colds. Rather low amounts of leukotrines, prostaglandins and histamine have been found in nasal secretions in colds (Callow et al., 1988) and blocking cholinergic transmission has limited effect on cold symptoms (Gaffey et al., 1988). On the other hand an inhibitor of mast cell degranulation, which impedes mediator release, has some beneficial effect on colds – even though this has not been proved to be due to an effect on mediators (Barrow et al., 1990a). It is important to do such studies carefully and objectively – for menthol which has long been used because it relieves the symptoms of nasal obstruction apparently does so by blocking the sensory pathway from the nose and leaving the patency of the airway unchanged. Methods are available to measure blood flow, mucosal temperature as well as patency and nasal secretion but these are only occasionally used to document the effects of virus infection on the nose (Bende et al., 1989) or the anticipated benefits of treatment.

However, it has recently been shown that bradykinin, which can induce the symptoms of nasal irritation and discharge, is found in raised concentrations in the nasal secretions of individuals with rhinovirus colds (Naclerio et al., 1987; Proud et al., 1990). As antikinins are now being developed it is reasonable to investigate whether these relieve the symptoms and signs of a cold, but an initial study of one candidate, NPC 567, has been unsuccessful (Higgins et al., 1991).

While it is attractive to approach the problem with specific hypotheses and specific probes it is also worth exploring less well-defined approaches to modifying the host response. In this area recent studies have shown that steroid

treatment delays the clinical response in experimental colds (Farr et al., 1990), and that local hyperthermia (inhaling water-saturated air at 43°C) has an immediate effect in improving symptoms but also a lesser effect that lasts a few days (Tyrrell et al., 1989). It would be interesting to determine what changes glucocorticoids or heat induce in the nasal mucosa and try to identify those that are associated with reduced symptoms. It is possible that 'stressing' nasal cells by hyperthermia turns on the so-called heat shock proteins and modifies the tissue response to infection.

The possible scope of treatment

In the early days of the Unit, some thought that antiviral treatment was intrinsically impossible, and others that it would be impractical because of the limitations to its use; for instance that it would need to be given prophylactically, or would have to be given intranasally. Nevertheless it is interesting that members of the first group of drugs shown to be effective against respiratory viruses in man, namely amantadine and its successors, *are* active when given therapeutically and by mouth. This seems to be because they have very advantageous pharmacokinetics – they are well-absorbed and secreted actively into the respiratory tract (e.g. Al-Nakib et al., 1986). On the other hand the particle-binding antirhinovirus drugs are very hydrophobic, which is probably the reason for their effectiveness, but it may also explain why they are not secreted with the mucus. As a result such molecules have to be given locally and the schedules of drug administration have to be quite demanding in order to keep the drug concentration high enough – they do not induce a long-lasting change like the antiviral state produced by interferon once it has attached to a cell. This indicates that antirhinovirus drugs with better pharmacokinetics, drug distribution and other properties might be more satisfactory clinically.

Even though better molecules are not yet available, it might still be possible to improve their performance by improving the formulation. In fact, in retrospect, the studies of dichloroflavan, the Roche chalcone and R61837 were not directly comparable, because although the dosage regimes, the challenge virus and the methods of observation were the same the drugs were formulated in different ways – in particular the Janssen drug formulation, in a cyclodextran, was distinctly and probably significantly different from that used in the earlier studies. It is probable that individual molecules of drug were located in the hydrophobic interior of what was externally a hydrophilic molecule. Thus it was more nearly in aqueous solution than the solution in oil or the micronized suspension of the other drugs.

Other methods of drug presentation are now being developed, for instance by incorporating them into small spheres of polymer which can both provide delayed release and have bioadhesive properties. This type of formulation made it possible to give insulin intranasally to rats and obtain sufficient absorption to have a significant metabolic effect.

This essay has focussed mainly on rhinoviruses, as the main cause of

common colds. However, we know that coronaviruses as well as paramyxoviruses or enteroviruses also make a small but significant contribution, and there is a substantial fraction due to as yet unidentified organisms (Larson et al., 1980). This means that even if a highly effective specific rhinovirus treatment were developed it would provide no benefit for many colds. Nevertheless the human coronaviruses are now being explored at the molecular level, and infection with one serotype can already be diagnosed by the use of a nucleic acid probe (Myint et al., 1990).

For this reason we should keep our options open and be prepared to study not only the antimediator treatments mentioned above but also ill-understood empirical treatments, such as local zinc treatment or nasal hyperthermia, both of which were presented originally as antiviral treatments. The former was administered as oral lozenges and seemed to produce some symptomatic benefit but had no effect on the virus infection in studies at our Unit; but good studies done elsewhere, using a slightly different virus and experimental design, showed no benefit. Local hyperthermia with air at 43°C and 100% relative humidity was originally suggested as a way to inhibit the replication of rhinoviruses. In a study in volunteers no inhibition was detected but in a study in general practice it seemed to produce symptomatic improvement as mentioned above.

Further studies

It is probably too much to hope for an antiviral that will attack all viruses, but a programme to search for an anticoronavirus drug might be well worth undertaking. Such a search might become more rational than in the past. Perhaps a specific binding site might be found in an essential virus protein, just as had been done for rhinoviruses.

Molecular techniques can now be used for rhinovirus detection, such as oligonucleotide probes for conserved regions of the 5′ non-coding region (Bruce et al., 1989) and now amplification by polymerase chain reaction (PCR) (Gama et al., 1989). Antibodies can be followed very sensitively and specifically by the use of ELISAs (e.g. Barclay et al., 1989). The host response can also be assessed by clinical and physiological methods. Tests of human performance enable us to assess the 'systemic' effects of the infection and the benefits of any treatment on this (Smith et al., 1988a). All this technology will enhance the detail in which the clinical study of drug effects can be documented, but unless or until an effective animal model can be developed it is my belief that experiments in human volunteers will continue to play a key part in the development of specific antiviral therapies.

Finale

This essay is expanded from a talk which was frankly a personal assessment of recent research work done at the Unit on antivirals against common colds. It

is, I am afraid, neither a complete history of common cold research in volunteers nor a full review of the results of recent work on anti-rhinovirus drugs. It certainly does not have the literary panache of one of the modern bioessayists. I hope it will be read and assessed for what it is, general views elicited from a jobbing biologist, who regards himself as very fortunate to have been paid to spend his working life in a fascinating area of research, and to have seen a drastic transformation of our understanding of viruses, disease and the possibility of treatment – and, as a bonus or by-product – has made many good friends around the world.

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