Progress towards control of the acute respiratory viral diseases of childhood*

C. R. PRINGLE

Many of the common respiratory illnesses of infancy and childhood are caused by viruses of the Paramyxoviridae family, in particular measles virus, respiratory syncytial (RS) virus and parainfluenzavirus type 3 (PI3). Effective measles vaccine was developed by classical methods, but these same methods have failed to provide vaccines to control RS and PI3 virus infections. The WHO Programme for Vaccine Development was initiated in 1983 to encourage the application of the new biotechnologies to continuing problems, such as the acute virus-induced respiratory diseases of childhood. At a meeting of research workers held in July 1986 under the auspices of this programme, renewed optimism was expressed concerning the prospects for immunoprophylaxis of RS virus-induced disease. Animal models are now available for evaluation of the immunogenic potential of candidate vaccines. Vaccinia/RS recombinant viruses have been produced which have allowed the immunogenic properties of individual RS virus proteins to be defined. Complete protection without the exacerbation of disease, which earlier had accompanied the use of formalin-inactivated vaccines, has been achieved in animals immunized with vaccinia virus recombinants expressing the F protein; partial protection was obtained using G protein gene vectors. PI3 appears to be an inherently stable virus and evidence from animal experiments suggests that bovine PI3 might be suitable for use as a live vaccine in man.

Communicable diseases of the respiratory tract are a major cause of morbidity and mortality throughout the world; they account for as many as 2.2 million deaths annually, infancy being the time of greatest risk. A considerable proportion of the common respiratory diseases of childhood are caused by viruses of the Paramyxoviridae family. The most troublesome of these viruses are measles virus, respiratory syncytial (RS) virus, and parainfluenzavirus type 3 (PI3).

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1 Chairman, Steering Committee on Acute Respiratory Viruses, WHO Programme for Vaccine Development. Professor of Virology and Head of the Virus Research Group, Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, England. Requests for reprints should be sent to this address
Measles virus infection, which can be controlled by immunization and appears to be near to eradication in some European countries, remains a problem principally in circumstances of social and economic deprivation. RS virus, on the other hand, is associated worldwide with epidemics of acute respiratory disease (bronchiolitis and pneumonia) in newborn infants, which occur annually with remarkable regularity in temperate zones. P13 is responsible for severe respiratory disease in young children, but the epidemics are less regular in occurrence than those caused by RS virus. Both these viruses are ubiquitous and sometimes cause upper respiratory tract infections in adults as well. RS virus infections, in particular, are also life-threatening in persons of old age.

Classical approaches have failed to yield effective vaccines to control infections due to both RS virus and P13. One of the five aims of the WHO Programme for Vaccine Development, which was initiated in 1983 to encourage research in neglected diseases, is the control of virus-induced acute respiratory diseases of childhood. Twenty-eight scientists\(^6\) associated with the Programme met on 26–27 June 1986 at the close of the American Society for Virology Conference in Santa Barbara, California, to review progress and exchange information. This meeting revealed that some of the initial research targets identified by the WHO Steering Committee for the acute respiratory viral diseases part of the vaccine development programme were close to achievement, and that the prospects for development of vaccines to control infections caused by RS virus and P13 had greatly improved since the inception of the programme.

VACCINES AGAINST RS VIRUS AND P13

Research has been concentrated on RS virus infections on account of their unique annual epidemicity, worldwide prevalence and defined target population, although work on P13 is accelerating and fast catching up. Until recently RS virus was as unfamiliar to mainstream virologists as to the man in the street, but interest and research have developed to the extent that, for the first time at an open scientific meeting, an entire session was devoted to RS virus alone.

In the two decades following the discovery of these viruses in the late 1950s and their association with respiratory disease in infancy, attempts to develop vaccines by conventional routes were unsuccessful. A formalin-inactivated RS virus vaccine had the effect of potentiating subsequent natural disease rather than conferring protection. This bitter experience effectively inhibited work on the development of inactivated vaccine. Subsequent attempts to develop a live RS virus vaccine at the U.S. National Institutes of Health (USNIH) were also abandoned because the candidate vaccines were considered to be either over-attenuated or to exhibit genetic instability.

Three recent developments, however, have revitalized the field and opened up new approaches to vaccine development. First, molecular cloning by synthesis of cDNA from RNA by reverse transcription has enabled the structure and sequence of the genomes of RS virus and the other paramyxoviruses to be determined. A consequence of the cloning and partial sequencing of the genomes of almost all the paramyxoviruses of medical importance is the recognition that RS virus is more complex and quite distinct from the other paramyxoviruses. Most of the individual genes of the A2 strain of RS virus have been cloned and in some cases their products have been expressed in vitro, allowing their immunogenic properties to be studied in isolation, uncomplicated by the presence of the other proteins of the virus.

Secondly, a development of equal significance has been the reinvestigation of the above-mentioned RS virus vaccine incident. Chimpanzees are the only animals which exhibit signs of illness similar to man; indeed, the first RS virus was isolated from a sick chimpanzee and, for a brief period, the virus was known by the name chimpanzee coryza agent. However, rodents are susceptible to RS virus infection, and it has recently been shown that the level of antibody necessary to provide passive protection in the cotton rat is very similar to the level of maternally-derived antibody in newborn children who are relatively more resistant to RS virus infection than infants a few weeks older. It has now been demonstrated by workers at the US NIH that the exacerbation of disease observed in the inactivated vaccine trials in children can be mimicked in cotton rats by administration of the same batch of vaccine used in the ill-fated trial. Analyses of responses in cotton rats have revealed that the formalin treatment used in preparation of the vaccine was undoubtedly responsible for the aberrant performance of the vaccine, and that enhanced antibody levels per se were not the determining factor. Consequently, there is no longer any constraint on the development and testing of inactivated vaccine. It has also been shown that passive immunization of animals with either polyclonal or monoclonal antibodies (anti-G or anti-P) can provide protection, and that the administration of antibody does not result in exacerbation of disease. At the present time, an intravenous gammaglobulin preparation with high RS virus neutralizing activity (Sandoglobulin) is under test in children in the USA.

Thirdly, the production of monoclonal antibodies to the structural proteins of RS virus and PI3 has enabled detailed studies of the variability of these viruses and their epidemiological behaviour to be initiated.

The state of progress in the development of vaccine against PI3, the next most important agent responsible for acute respiratory illness in infancy, was also considered by the participants at the meeting. The results of the sequencing of the genomes of a single strain propagated independently in three different laboratories suggested that PI3 was an inherently stable virus. Evidence from protection experiments in cotton rats, squirrel monkeys and chimpanzees suggested that bovine PI3 might be suitable for use as a live vaccine in man.

NEW STRATEGIES FOR VACCINE DEVELOPMENT

The meeting also focused attention on new strategies for vaccine development. The subjects covered ranged from discussion of the nature of the genomes of paramyxoviruses to consideration of recent seroepidemiological studies.

The genomes of all the paramyxoviruses are uninterrupted linear sequences with very little informational overlay. In contrast to segmented genome viruses (e.g., influenza virus), the exchange of genes by reassortment of genome subunits is not possible in paramyxoviruses and variation can only originate by mutation. The genome of RS virus is a linear array of 10 genes, whereas the paramyxoviruses have only 6 or 7 genes. In RS virus two non-structural genes are interposed between the nucleoprotein (N) gene and the 3' terminus, which is a pattern not found in any other negative-strand RNA virus. At the other end of the genome there is an unusual 68 nucleotide overlap of the end of the penultimate 22K membrane protein coding gene and the start of the presumptive polymerase (L) gene, which is another distinctive feature of RS virus. The nucleotide sequences of nine of the ten genes of the A2 strain of RS virus have been determined and in only a single gene is there any hint of homology with genes coding for proteins of similar

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6 Sandoz Inc., East Hanover, NJ, USA.
function in other paramyxoviruses. RS virus, although morphologically similar, is not closely related to the other paramyxoviruses. The paramyxoviruses themselves show a degree of variation in genome structure with respect to the presence of overlapping coding sequences and of variability in the extent of the non-coding intergenic regions.

Current research is confirming that the immunologically important proteins are the viral envelope glycoproteins. All the paramyxoviruses have two membrane glycoproteins but those of RS virus are distinctive. Glycoprotein F of RS virus is functionally analogous to the fusion proteins of the other paramyxoviruses, and glycoprotein G of RS virus is the presumptive attachment protein. The G protein is exceptional in that possibly more than half the relative molecular mass of the protein is accounted for by sugar residues predominantly attached by O-linkages, whereas in most virus proteins the polysaccharides are attached by N-linkages. The structure and synthesis of this unusual protein have still to be worked out unequivocally; a plausible hypothesis is that the mature G protein is a less heavily glycosylated dimeric structure. The role of glycosylation in the antigenicity of this protein has not yet been investigated.

The cloned genes coding for both of these RS virus proteins have been recombined into vaccinia virus vectors independently by research groups at the USNIH and at the University of North Carolina, Chapel Hill, NC, USA. The products of the F and G genes were expressed in a functional form in cells infected by these recombinant vaccinia viruses. This provides a flexible and elegant experimental system whereby the immunogenic and protective potential of these proteins can be investigated individually. A similar result can be achieved by a different route. Preparations of the F and G proteins can be isolated from disrupted purified virions or from infected cell extracts by affinity chromatography using monoclonal antibodies of the appropriate specificity. It is clear from the results of such experiments that either of these two membrane glycoproteins is able to confer protection in animals. No additive effect is observed if the F and G proteins are used together; a single protein vaccine (or perhaps ultimately a synthetic peptide vaccine) is therefore a feasible proposition. Experiments with vaccinia virus recombinants also suggest that glycoprotein F may have better prospects as a single protein vaccine since a recombinant vaccinia virus expressing the RS virus G protein failed to confer protection when the challenge virus belonged to a different monoclonal antibody-defined subtype.

Only minor antigenic differences have been detected among different isolates of RS virus using polyclonal animal or human antisera. However, comparisons of strains using batteries of monoclonal antibodies have revealed the existence of at least two subtypes. Work in Sweden, the United Kingdom and the USA has revealed systematic differences in the electrophoretic mobilities of the F and P proteins of viruses of the two subtypes. Epidemiological studies indicate that both subtypes occur worldwide and furthermore they cocirculate in the same epidemics. Preliminary results of monitoring of the distribution of subtypes in the Boston area (in the USA) during winter epidemics over the past two years suggest that progressive changes in frequency of the subtypes may occur during the course of an epidemic with local discontinuities in the occurrence of subtypes. The relative frequencies of the subtypes did not correlate with the severity of disease outbreaks, and the epidemiological significance of the subtypes is still unclear.

Comparison of the reactivity of human sera suggested that the G protein of RS virus had diverged more than the F protein, whereas experiments with sera from cotton rats yielded the reverse conclusion. This indicated that the glycoprotein epitopes were seen differently by different host organisms. Consequently the results of animal experiments must be interpreted with caution and may not be directly applicable to man. None the less the availability of animal models will facilitate vaccine development.

The immune response to infection in terms of the specificities of antibodies varies between individuals and with age. Work in the United Kingdom has shown that children under six months of age were unable to induce an IgG1 response, whereas IgG2, IgA and
IgM responses were induced. The amounts of IgG4 in adults were no greater than normal. Screening of the sera of 400 pregnant women for antibody to four different epitopes on the F protein revealed considerable variability in responses between individuals. Investigations in the USA have shown that younger children made less antibody than older children, and that the G protein appeared to be less immunogenic in young children (under 7 months of age). Study of the response of children to reinfection is under way in the USA.

It has been shown in the mouse that T cells are important in the clearance of RS virus, and that different subsets of T lymphocytes vary in their responsiveness to RS virus antigens. Vaccinia/RS virus recombinants have been utilized to produce target cells expressing individual RS virus proteins. Preliminary experiments in the mouse indicated that the RS virus nucleoprotein rather than the surface G protein was recognized by cytotoxic T cells. This finding, though at first surprising, is similar to the more extensive observations on the influenza viruses.

FUTURE PROSPECTS

A forward look at the prospects for the development of an effective RS virus vaccine was given by Dr R. M. Chanock, who some thirty years previously had first associated this virus with acute respiratory disease in infancy. Dr Chanock expressed renewed optimism regarding the prospects for immunoprophylaxis of RS virus-induced disease. The animal models now available will enable the immunogenic potential of candidate vaccines to be evaluated and, more importantly, to detect any exacerbation of disease. The performance of the vaccinia/RS virus recombinants in animals, whereby complete protection was provided by F gene vectors and partial protection by G gene vectors, was promising. The vaccinia virus vectors could be used to investigate some of the remaining unknown factors, such as the possible complication of vaccine-induced immunosuppression (since there was some evidence that maternal antibody depressed the anti-G response), and whether preexisting anti-RS virus antibody would inhibit replication of the recombinants. Although the vaccinia virus currently employed in construction of recombinants is neuroviralent, it should be possible to transfer the RS virus genes into a non-neurotropic vaccine strain. Further improvements in vector technology are required to ensure an adequate immune response in primates.

Despite the revelation of antigenic heterogeneity by monoclonal antibody studies, the present evidence indicates that this variation is not clinically important. Nevertheless the apparently random occurrence of the subtypes perhaps indicates that more epidemiological data are required. Therefore the clinical significance of subtypes should not be prejudged. An effective vaccine might need to be compounded from more than one component.

Significant achievements have been made, such as the protection by passive immunization of animals without exacerbation of disease and the safe administration to children of an intravenous gammaglobulin preparation with high RS virus neutralizing activity. The potential of passive immunotherapy has been enhanced by recent work which suggested that protection conferred by topical application was as good as or better than that after injection. Research on paramyxovirus vaccines is now in an exponential phase of growth and the prospects for control of the acute respiratory diseases caused by these viruses are greatly enhanced.