ARCHIVES OF DISEASE IN CHILDHOOD

*The Journal of the British Paediatric Association*

## *Annotations*

# Respiratory syncytial virus – how soon will we have a vaccine?

It is a quarter of a century since the first attempts to develop vaccines to respiratory syncytial virus ended in failure. Neither formalin inactivated nor live virus vaccines, administered intramuscularly, provided significant protection of infants and both have been associated with enhanced rather than reduced severity of disease on subsequent natural infection.[1 2] After 25 years of intensive research, once again, a number of candidate vaccines are being considered for clinical trial. Has our understanding of the mechanisms of disease enhancement and the poor protection afforded by the early vaccines advanced far enough to allow these trials to be pursued with confidence?

### Disease enhancement

The immunological events leading to enhancement must be studied predominantly in animal models.[3] Passive immunisation with either polyclonal or monoclonal antibodies does not potentiate disease on subsequent virus challenge in either animals or in human infants.[4] T cells, however, may be implicated as, in mice, passive administration or respiratory syncytial virus specific T cells of both $CD4^+$ and $CD8^+$ phenotypes exacerbates disease on subsequent live virus challenge.[5] The response to formalin inactivated vaccine in the lungs of vaccinated mice has been shown to be dominated by a type 2 T helper cell response, previously associated with disease enhancement rather than protection in a number of other murine infections including leishmaniasis.[6] Damage can be prevented by depletion of $CD4^+$ cells.[7] The relevance of these findings to human vaccine derived disease will be difficult to assess further. However, it is noteworthy that the children vaccinated with formalin inactivated virus 25 years ago made an unexpectedly brisk T cell proliferative response to respiratory syncytial virus antigens.[8]

### Protective immunity

The ability of serum neutralising antibody to protect infants against respiratory syncytial virus bronchiolitis has been demonstrated by passive immunisation with intravenous immune globulin.[9] In cotton rats, prophylactic human immune globulin is protective when serum neutralising antibody levels of 1:350 are achieved. Extrapolation of these promising results in rodents to human infants was initially thwarted as it proved difficult to achieve the necessary high level of serum neutralising antibody with available immunoglobulin preparations. This problem may have been overcome by the development of respiratory syncytial virus enriched immunoglobulin (RSVIG), gammaglobulin prepared from a pool of sera selected for high antirespiratory syncytial virus titre in a microneutralisation test. Monthly administration of RSVIG to infants and children at high risk of severe illness with respiratory syncytial virus produced serum antibody titres of 1/200 to 1/400 and some evidence of reduced lower respiratory tract infections, hospitalisations, and days in hospital.[4] A further trial is in progress.

Following from these studies it is reasonable to postulate that the failure of early vaccines may have resulted from their inability to generate sufficient functional antibody. Neutralising antibody levels achieved by both live and killed vaccine were well below the 1/200 found necessary for effective prophylaxis.[10 11] Why should this be so? Subsequent work has shown that formalin inactivated virus induces neutralising and fusion inhibiting antibody poorly.[12 13] The major epitopes responsible for the generation of these functional antibodies are dependent upon the conformation of the F or fusion glycoprotein of the virus,[14] which may have been disrupted during preparation of the vaccine. For the live virus vaccine, poor responses have been attributed to the immunosuppressive effect of residual transplacentally acquired antibody present in the serum of younger infants (reviewed by Toms[15]).

### Antigenic variation

Antigenic variation is a further factor which may have reduced the efficacy of the early vaccines. Respiratory syncytial virus isolates fall into two subgroups A and B, and genetic heterogeneity both between and within these subgroups is well defined and most pronounced in two of the three surface glycoproteins, the attachment protein G and the small hydrophobic protein, SH.[16 17] Early work established that field isolates of respiratory syncytial virus also vary antigenically when compared in neutralisation tests against animal sera.[18] More recently, monoclonal antibodies have revealed strain and subgroup specific antigenic variation, not only in the variable G glycoprotein, but

also in the relatively well conserved third glycoprotein, F.[14 16 19] Despite this in vitro evidence of variation in both F and G, which are the major protective antigens of the virus, small animals immunised with virus of one subgroup, or the F glycoprotein purified from it, develop immunity against infection of the lung with virus of both subgroups. Immunisation with the G glycoprotein alone produces only partial protection against the heterologous subgroup.[20 21] However, such studies have been carried out only with 'prototype' virus strains in rodents, which respond well to respiratory syncytial virus antigens and have relatively low susceptibility to the virus. The heterogeneity of field isolates of virus may be more important in more susceptible human beings who are more difficult to immunise. There is some evidence that this is so as, in infants, neutralising serum antibody responses to subgroup A and B viruses are significantly reduced against strains of the heterologous subgroup.[22] Furthermore, infection with a virus of one subgroup appears to render reinfection with that subgroup less likely, although the numbers of double infections studied have been small.[23]

These studies suggest that modern candidate vaccines must induce a 'protective' rather than a disease potentiating immune response, including high levels of neutralising antibody, despite immunosuppressive transplacental antibody, and a wide spectrum of immunity capable of dealing with a wild virus population of ill defined heterogeneity. How well do they fulfil these criteria?

## Subunit and live recombinant vaccines

Subunit vaccines, comprised either of the glycoproteins purified from the virus or an F/G chimera created by recombinant DNA technology, have proved capable of inducing high levels of neutralising antibody and protection in rodents but are less immunogenic in more permissive primates.[3] Worryingly, in mice, or in rats if time is allowed for immunity to wane, inflammatory changes occur in the lungs of vaccinated animals on live virus challenge, although disease enhancement is less than with formalin inactivated preparations. Recombinant vaccinia viruses and adenoviruses designed to express respiratory syncytial virus glycoprotein genes during infection of the respiratory mucosa, circumventing immunosuppression by transplacental antibody in the serum and inducing both mucosal and systemic immunity, have achieved similarly disappointing results (reviewed by Murphy *et al*[3]).

Despite this, trials of a purified glycoprotein preparation have been carried forward on children but only those with prior experience of respiratory syncytial virus infection. Vaccinated infants developed a rise in serum neutralising antibody and were significantly less likely to contract respiratory syncytial virus infection during their first, but not their second epidemic after vaccination. There was no evidence of immunopotentiation even in the absence of significant protection.[24] These results are encouraging but it would be unwise to assume that immunisation of seronegative infants would induce a similarly benign immune response. It is also unlikely that this vaccine would be sufficiently immunogenic in the unprimed infant in the presence of transplacentally acquired antibodies and by no means certain that it will confer equivalent protection against all circulating strains of the virus.

## Live attenuated vaccines

Early attempts to attenuate respiratory syncytial virus by adaptation to replication at low temperature in cell cultures or the induction of temperature sensitive mutations produced inadequately attenuated or genetically unstable strains of virus but revealed no evidence of exacerbated disease on subsequent, natural infection.[1] Continuing efforts have thus been made to develop stable, immunogenic, and attenuated strains of respiratory syncytial virus assessed in adult volunteers and in seronegative chimpanzees. The most promising strains currently available are derived from a cold adapted virus by one or two rounds of chemical mutagenesis followed by selection for temperature sensitivity. These strains are genetically stable on prolonged infection of nude mice and both attenuated and immunogenic in highly susceptible seronegative chimpanzees inducing high levels of neutralising serum antibody and protection against challenge.[3]

Excitement over these results must be tempered by the experience of the last quarter century. Protection of animals in the laboratory is much more easily achieved than protection of infants against natural infection. Repeated lower respiratory tract infection in the first years of life is the norm and this has not yet been adequately explained. The discovery that chimpanzees, and by implication human beings, are more permissive to the virus and less able to respond successfully to virus antigens than rodents may help to explain past failures and inspires confidence in these vaccines. The natural route of immunisation, via the mucosa, may reduce the immunosuppressive effect of transplacentally acquired antibody. However, a further major difference between the laboratory and the nursery lies in the nature of the challenging virus. Infants are exposed to a population of viruses the antigenic heterogeneity of which we are only just beginning to perceive. The game we are playing with respiratory syncytial virus is not yet over and antigenic heterogeneity may prove to be its ace in the hole.

G L TOMS

*Department of Virology,*
*University of Newcastle upon Tyne,*
*The Medical School,*
*Framlington Place,*
*Newcastle upon Tyne NE2 4HH*

1 Stott EJ, Taylor G. Respiratory syncytial virus: brief review. *Arch Virol* 1985; **84:** 1–52.
2 Kimman TG, Sol J, Westenbrink F, Straver PJ. A severe outbreak of respiratory tract disease associated with bovine respiratory syncytial virus probably enhanced by vaccination with modified live vaccine. *Vet Q* 1989; **11:** 250–3.
3 Murphy BR, Hall SL, Kulkarni AB, *et al.* An update on approaches to the development of respiratory syncytial virus (RSV) and parainfluenza virus type 3 (PIV3) vaccines. *Virus Res* 1994; **32:** 13–36.
4 Groothius JR. The role of RSV neutralizing antibodies in the treatment and prevention of respiratory syncytial virus infection in high-risk children. *Antiviral Research* 1994; **23:** 1–10.
5 Alway WH, Record FM, Openshaw PJM. $CD4^+$ T cells clear virus but augment disease in mice infected with respiratory syncytial virus. Comparison with the effects of $CD8^+$ T cells. *Clin Exp Immunol* 1992; **88:** 527–36.
6 Graham BS, Henderson GS, Tang Y-W, Lu X, Neuzil KM, Colley DG. Priming immunization determines T helper cytokine mRNA expression patterns in lungs of mice challenged with respiratory syncytial virus. *J Immunol* 1993; **151:** 2032–40.
7 Connors M, Kulkarni AB, Firestone C-Y, *et al.* Pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of $CD4^+$ T cells. *J Virol* 1992; **66:** 7444–51.
8 Kim HW, Leikin SL, Arrobio J, Brandt CD, Chanock Rm, Parrot RH. Cell-mediated immunity to respiratory syncytial virus induced by inactivated vaccine or by infection. *Pediatr Res* 1976; **10:** 75–8.
9 McIntosh K. Respiratory syncytial virus – successful immunoprophylaxis at last. *N Engl J Med* 11993; **329:** 1572–3.
10 Kim HW, Canchola JG, Brandt CD, *et al.* Respiratory syncytial virus disease in infants despite prior administration of antigenic inactivated vaccine. *Am J Epidemiol* 1969; **89:** 422–34.
11 Buynak EB, Weibel RE, Carlson AJ, McLean AA, Hilleman MR. Further investigations of live respiratory syncytial virus vaccine administered parenterally. *Proc Soc Exp Biol Med* 1979; **160:** 272–7.
12 Murphy BR, Prince GA, Walsh EE, *et al.* Dissociation between serum neutralizing and glycoprotein antibody responses of infants and children who received inactivated respiratory syncytial virus vaccine. *J Clin Microbiol* 1986; **24:** 197–202.
13 Murphy BR, Walsh EE. Formalin-inactivated respiratory syncytial virus vaccine induces antibodies to the fusion glycoprotein that are deficient in fusion-inhibiting activity. *J Clin Microbiol* 1988; **26:** 1595–7.

14 West WHL, Lounsbach GR, Bourgeois C, *et al.* Biological activity, binding site and affinity of monoclonal antibodies to the fusion protein of respiratory syncytial virus. *J Gen Virol* 1994 (in press).
15 Toms GL. Vaccination against respiratory syncytial virus: problems and progress. *FEMS Microbiol Immunol* 1991; **76:** 243–56.
16 Anderson LJ. Strains of respiratory syncytial virus: implications for vaccine development. In: de la Maza LM, Peterson EM, eds. *Medical virology 9.* New York: Plenum Press, 1990: 187–205.
17 Cane PA, Matthews DA, Pringle CR. Analysis of relatedness of subgroup A respiratory syncytial viruses isolated worldwide. *Virus Res* 1992; **25:** 15–22.
18 Beem M. Repeated infections with respiratory syncytial virus. *J Immunol* 1967; **98:** 1115–22.
19 Robinson BS, Everson JS. Epitopic specificities of human serum antibodies reactive with respiratory synctial virus fusion protein. *Arch Virol* 1992; **125:** 273–86.
20 Prince GA, Horswood RL, Koenig DW, Chanock RM. Antigenic analysis of a putative new strain of respiratory syncytial virus. *J Inf Dis* 1985; **151:** 634–7.
21 Johnson PR, Olmsted RA, Prince GA, *et al.* Antigenic relatedness between glycoproteins of human respiratory syncytial virus subgroups A and B: evaluation of the contributions of F and G glycoproteins to immunity. *J Virol* 1987; **61:** 3163–6.
22 Hendry RM, Burns JC, Walsh EE, *et al.* Strain specific serum antibody responses in infants undergoing primary infection with respiratory syncytial virus. *J Inf Dis* 1988; **157:** 640–7.
23 Mufson MA, Belshe RB, Orvell C, Norrby E. Subgroup characteristics of respiratory syncytial virus strains recovered from children with two consecutive infections. *J Clin Microbiol* 1987; **25:** 1535–9.
24 Tristram DA, Welliver RC. Respiratory syncytial virus vaccines: can we improve on nature? *Pediatr Ann* 1993; **22:** 715–8.

# Fragile X syndrome

The past few years have witnessed dramatic advances in our understanding of the fragile X syndrome. Clarification of the underlying molecular abnormality at the gene level[1] has enabled the development of more sensitive and specific diagnostic tests based on Southern blot and polymerase chain reaction technology.[2] Increasing definition of the associated behavioural phenotype is of potential use diagnostically and therapeutically.[3–5] Also, specific problems associated with female carrier status are being increasingly recognised.[6 7]

## Epidemiology

Many paradoxes persist, however, along with ethical dilemmas surrounding the issue of whether or not to undertake presymptomatic screening for an as yet incurable disorder, and if so on which populations this should be undertaken. The condition is certainly common,[8] although exact prevalence statistics vary considerably.[9] However, it is still generally acknowledged as the most common inherited cause of learning disabilities affecting between one in 1000 and one in 3000 individuals. Some authorities even speculate that once the proportion of affected individuals who are identified increases, the frequency may even match that of Down's syndrome.[10]

Recent audit of a regional genetic service's pick up of fragile X cases found a substantial shortfall compared with expected numbers based on reported prevalence rates.[11] The authors felt that regional variation in prevalence rates was unlikely to be the explanation. Sensitivity problems with the more traditional chromosomal diagnostic analysis may have played a part[12] but it was concluded that the most likely reason was under-referral of appropriate cases for testing. This view is supported by evidence that clinicians who refer individuals for fragile X testing with a suspicion based on behavioural features are usually wrong (J Turk, unpublished data). Thus where selection criteria are being used to determine which children to refer to genetic services, they are presumably often inappropriate. This implies that either more children should be tested or that criteria for genetic referral need to be improved.

## Genetics

The fragile X mental retardation gene (FMR-1) is located at the Xq27.3 'fragile' locus and consists of multiple CGG trinucleotide repeats.[1] Transgenerational progression from premutation in normal transmitting males and their daughters to full mutation in grandchildren is explained by progressive expansion of the CGG repeat in the 5′ untranslated region of the FMR-1 gene. This expansion produces abnormal DNA hypermethylation and disturbed protein synthesis.

Normal X chromosomes have approximately six to 45 CGG repeats with most clustering around 30 (D Nelson, 8th International Congress of Human Genetics, Washington, 6–11 October 1993). In the general population, 1% may have a small CGG expansion which represents carrier status and is known as a 'premutation'. These heterozygous premutation carriers have an expansion of between 52 and 200 triplet repeats that is unstable on maternal transmission, with the chance of progression to a full mutation reaching 100% for women with a premutation of 90 repeats or above.[13 14] In the full mutation, repeat sequences exceed 200 triplets in size. Normal transmitting males have length increases of about 500 base pairs. Affected males have an increase of between 500 and 5000 base pairs, with hypermethylation of the site.[15] It should be noted that these figures represent *base pairs* and not *CGG repeats*. The reason for this was the use in the study of probes to detect very localised DNA rearrangements that constituted the fragile X mutations, and whose target was a 550 base pair GC-rich fragment. The insertion size in females may relate to clinical severity but this has yet to be confirmed.[15] It has also been demonstrated that amplification of the CGG repeat blocks FMR-1 gene transcription, which results in absence of the FMR protein.[16] Efforts are under way to identify the protein whose production is interfered with by the fragile X mutation. The protein's function is as yet unknown, although it has been postulated to be of importance in gene regulation.[1]

Fragile X variants have been identified. These manifest with a similar hypochromic fragile site cytogenetically. However, the molecular abnormality is elsewhere. A common fragile site[17] is located at Xq27.2 and has been assigned FraX-D to distinguish it from fragile X syndrome (FraX-A). This site is a potential source of misdiagnosis in female carriers, prenatal testing, and occasional males with very low levels of fragile site expression.[18] Its prevalence in the general population is unknown, but it is not thought to be associated with mental retardation. High quality banded cytogenetic preparations have confirmed a fragile site distal to the FMR-1 gene, in the proximal portion of Xq28.[18] This site (FraX-E) is folate sensitive. Cytogenetically it is virtually indistinguishable from FraX-A. The molecular abnormality in FraX-E does appear to expand through generations though perhaps not as consistently as in FraX-A.[19] Mental retardation is usually mild. Individuals have amplifications of a GCC repeat.[20] A third fragile site, FraX-F, has been identified in Xq27-q28.[21] Using fluorescent in situ hybridisation (FISH) the site has been shown to lie distal to FraX-A and