Vaccination against respiratory syncytial virus: problems and progress

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Received 19 November 1990
Revision received 26 April 1991
Accepted 21 May 1991

Key words: Respiratory syncytial virus; Vaccine; Immunity; Antigenic variation; Hypersensitivity; Epitope mapping

The respiratory syncytial (RS) viruses belong to the pneumovirus genus of the paramyxoviridae [65]. The virus genome is a single strand of negative sense RNA which is transcribed into ten major species of messenger RNA, each of which encodes a viral protein. Three glycoproteins are associated with the surface of the virion and the infected cell — the attachment protein, G, the fusion protein, F, and a small hydrophobic protein of unknown function, SH or 1A. Two proteins are associated with the membrane, M1 and M2 or 22K. The major nucleocapsid protein, N, is associated with a phosphoprotein, P, and the putative polymerase protein L. In addition there are two non-structural proteins of, as yet, unknown function [65].

Human isolates fall into two antigenic subgroups, A and B, and antigenically distinct viruses have been recovered from both cattle and goats [92]. In human beings primary infection is generally contracted during the first two years of life and a high incidence of severe lower respiratory tract disease is associated with infection between one and six months of age [31,62]. Infants with congenital heart disease, bronchopulmonary dysplasia or immune dysfunction are particularly at risk [33,35,61]. Reinfection is commonplace but disease severity decreases with increasing age and frequency of exposure [32,40]. Bovine RS virus infection of calves, although less intensively studied, resembles the human disease in many respects [107].

The incidence of RS virus reinfection remains high in older children [20] and adults and may be associated with otitis media [55] and severe lower respiratory tract infection in the immunocompromised [23,78] and the aged [100]. However, the major impact of RS virus infection is upon the
very young and it is for this group that a vaccine is most urgently required.

Conventional vaccines

In the first attempt to develop an RS virus vaccine a human virus strain, concentrated from monkey kidney cell cultures, was inactivated with formalin, adsorbed to alum and administered intramuscularly. Although three doses of this inactivated vaccine induced rises in neutralising and complement fixing antibodies in the majority of infants vaccinated, the attack rate on subsequent exposure to natural virus epidemics was not reduced. Moreover, this vaccine pre-disposed infants to severe lower respiratory tract disease on subsequent infection with the virus (review [108]). The possible mechanisms leading to this potentiating effect are discussed below.

Attention thereafter shifted to the development of live virus vaccines. A clinical isolate of virus passaged ten times in diploid lung fibroblasts and administered subcutaneously produced good immune responses including neutralising antibody in seronegative children. In infants, however, responses were poorer and this was attributed to inhibition by maternally acquired antibody. This vaccine was also ineffective, the incidence of natural infection in infants and children following vaccination being similar in vaccine and placebo groups [11].

Two approaches to the development of attenuated strains of the virus which can be administered by the natural route of infection have also been extensively explored but without success to date. Cold adapted viruses, although attenuated in adult volunteers, retain residual virulence for infants. Temperature-sensitive mutants have also been developed which are avirulent but immunogenic and protective in adults. However, such mutants have so far proved unacceptably pathogenic and also genetically unstable on primary infection of infants (review [108]).

In cattle the conventional approaches to vaccine development have met with more success. Although formalin-inactivated virus was unsuccessful in effecting protection, a gluteraldehyde-fixed nasal mucosa cell line persistently infected with RS virus in a Quil A adjuvant was found to induce serum and mucosal antibody responses, lymphocyte transformation responses and was protective. None of over 400 animals vaccinated has shown signs of enhanced disease on experimental or natural exposure to the virus [113].

Live attenuated virus administered by an intramuscular route has also been shown to be immunogenic and protective in calves although not in all trials [22,108]. One possible reason for the lack of consistency in the performance of this vaccine may be the influence of variable levels of maternal antibody which also reduces the immunogenicity of the equivalent human vaccine in the very young. Although this type of vaccine has proved to be immunogenic and non-toxic in the majority of animals [22], Kimman et al. [53] recently reported severe or fatal disease in animals vaccinated during a naturally occurring outbreak of bovine RSV infection, but not in unvaccinated animals with serological evidence of infection housed in the same building. The significance of this report is unclear and further investigations into the aetiology of the outbreak are warranted.

Experience with this first generation of vaccines has thus highlighted several problems which must be overcome if useful immunity is to be developed in human infants around the first month of life. Firstly, it has proved difficult to protect infants with vaccines which appeared adequate in older children and adults. Secondly, there is the alarming possibility that immunisation may potentiate rather than ameliorate disease on subsequent exposure to the virus In addition to these problems, it is unclear what effect antigenic variation, recently revealed by monoclonal antibody studies, may have upon the efficacy of future vaccines.

Engendering protective responses in the very young

The peak incidence of bronchiolitis following RS virus infection in human infants comes at two months of age. Inducing effective protection of the respiratory tract in infants as young as this is a formidable task. Even primary natural infection does not engender satisfactory immunity and up to 75% of children infected in their first winter
may be reinfected in their second with a substantial incidence of lower respiratory tract disease [32]. Subsequent reinfections do, however, result in a degree of protection. A successful vaccine would ideally improve upon natural infection in the induction of immunity. To design such a vaccine it is necessary to unravel which elements of the immune response can contribute to protection and to investigate why infants may have difficulty in making these responses.

**Serum antibody**

At first sight it might be supposed that serum antibody plays a minor role in protection as most infants in hospital with RS virus have become infected despite the presence of transplacentally-acquired antibody in their blood. Several lines of evidence, however, indicate that high levels of serum antibody are helpful. In animal studies virus replication in the lung can be reduced by high titre antibody administered passively [37,97]. In human beings, severe disease is less common in the first month of life when antibody levels are highest [81], and two groups have found that high maternal antibody levels correlate with a lower incidence of infant infection [31,86]. It is necessary to resolve whether this reflects a direct protective effect upon the infant or reduced exposure of infants whose mothers are immune. Studies of infants in hospital have provided little support for a link between transplacentally-acquired antibody, measured by complement fixation test, neutralisation test or ELISA, and the severity of RS virus disease which might be expected if antibody played a direct role [13,57,115]. However, in a community study, Kasel et al. [49] have found higher serum antibody levels in infants with upper respiratory tract rather than lower respiratory tract disease indicating that transplacentally acquired antibody may protect the lung.

Reinfetion rates are also inversely correlated with serum neutralising antibody titres [24,32], and children who become reinfected have lower antibody levels than their peers who do not. This suggests that protection depends upon developing immunity rather than increasing physiological maturity of the lung as the children age [49].

Vaccine induction of protective serum antibody responses in young infants may prove difficult. Infants under six months of age produce poor serum antibody responses following natural RS virus infection [93,124]. Recent studies in calves indicate that this may result from an immunosuppressive effect of residual maternal antibody rather than immunological immaturity of the infant. It is difficult to assess the effect of maternal antibody, which is of the IgG class, upon the infants’ IgG response. However, in calves allowed to acquire maternal antibody through colostrum, both serum and mucosal IgA and, to a lesser extent, IgM antibody responses were suppressed when compared to colostrum deprived animals. Nonetheless, both groups of animals were equally protected when re-challenged after infection, indicating that factors other than high serum or mucosal antibody levels may also contribute to protection [52]. There is also evidence of immunosuppression by maternal antibody in human infants. Thus Murphy et al. [72] found that poor IgA responses to the viral attachment glycoprotein (G) correlated with pre-existing maternal antibody levels. Toms et al. [115] observed reduced IgM responses to infection in the very young but these did not correlate with residual maternal antibody at the acute stage of infection. Further work is needed to determine the mechanism by which residual antibody blocks the infant response in the hope that it may be possible to devise a strategy to circumvent the problem.

**Mucosal immunity**

Definitive evidence that a secretory antibody can protect infants against lower respiratory tract infection is lacking. It is unclear, therefore whether vaccines inducing both local and systemic responses are to be preferred. In adult volunteers, neutralising antibodies in the nasal secretions, mainly IgA, were associated with reduced virus shedding and upper respiratory tract disease following RS virus challenge [66,67]. In young infants, cessation of virus shedding in the
nasal secretions coincides with the appearance of sIgA antibodies [64]. However, this may represent interference with virus isolation rather than virus clearance. These observations pertain to upper respiratory tract infection and there is little evidence that nasal antibody protects infants against lower respiratory tract disease. No correlation has been found between acute nasal antiviral IgA levels or the speed of the response and disease severity among children in hospital (Toms et al., unpublished results; [50]) but further longitudinal community studies are required.

Satisfactory induction of local immunity in the very young may prove difficult. Nasal IgA responses are low and transient following primary infection in infants under six months of age and correlations with neutralising activity are poor [63]. In older infants, or on reinfection, antibody levels are increased and persist longer [50].

Passive protection

The evidence that antibody may mediate protection against infection has suggested the possibility of passive immunisation, which might avoid some of the pitfalls of vaccination of the infant. This might be effected indirectly by immunisation of the mother during pregnancy with subsequent transfer of antibodies to the infant via placenta and colostrum. Alternatively, antibody preparations might be administered directly either intravenously or topically to the infant’s respiratory tract.

Immunisation of pregnant women presents a safety hazard which would be difficult to assess fully. In addition, studies of natural infection with RS virus during pregnancy have indicated a degree of immunosuppression in the second and third trimesters which prevented significant boosting of serum antibody levels [79]. Despite this, colostra IgA antibody levels were raised in mothers exposed to RS virus during pregnancy. Breast feeding has been shown to reduce the admission of infants to hospital with RS virus infection [101]. However, there is no evidence linking this to passive acquisition of colostral IgA antibody and Nandapalan et al. [80] were unable to demonstrate any correlation between the anti-RS virus IgA antibody present in breast milk and protection of the breast-fed infant.

Intravenous administration of human immunoglobulins containing neutralising antibodies has been assessed both for therapy and prophylaxis of RS virus infection in rodents. In owl monkeys and infected human infants similar preparations have been used therapeutically [39]. In all cases significant reductions in virus replication in the lower respiratory tract were observed. Topical administration into the trachea in cotton rats and monkeys achieved similar results with lower doses of antibody. This approach, therefore, holds considerable promise and clinical trials to determine the efficacy of prophylaxis with intravenous immunoglobulin in infants at high risk from RS virus have been proposed. Progress may be greatly facilitated by the recent development of ‘humanised’ murine monoclonal antibodies in which the specificity determining hypervariable regions of the gene coding for a murine antibody capable of neutralising RS virus have been engrafted by genetic manipulation into a gene coding for a human antibody. If found to be effective in vivo, a pool of several such antibodies could form a safe and plentiful source of prophylactic with a high specific activity.

Cell mediated immunity

The importance of T lymphocytes in recovery from RS virus infection has been suggested by the severity of disease in patients with congenital T cell defects [25]. Whilst it is clear that a vaccine must adequately stimulate T helper cell responses, a cytotoxic T cell response is currently regarded as a two-edged sword capable of both speeding clearance of the virus infection and contributing towards an inflammatory response which may impair function in the infant lung. Virus-specific, class 1 HLA restricted, cytotoxic T lymphocytes have been demonstrated in the blood of adults during the RS virus epidemic season [7,9,16]. Similar virus-specific cytotoxic activity has been demonstrated in peripheral blood from infants with RS virus infection although HLA restriction was not demonstrated in these studies [16,45]. Activity is confined to a minority of in-
fants early after infection and declines rapidly. Numbers so far examined are too small to establish links with severity of infection and evidence regarding an increase in reactivity with age is contradictory.

In mice, administration of monoclonal antibody directly to infants or to the mother during gestation can lead to suppression of a CTL response in the infant on neonatal infection with the virus. This suggests that the infant’s cell mediated response, like the antibody response, may be suppressed by passively acquired maternal antibody [6].

The development of the mouse model has allowed a direct assessment of the role of cytotoxic T cells in the control of infection [5,8,9]. In this system it has been demonstrated that passive transfer of immune T cells can clear virus from the lungs of T cell deficient and normal mice [14,15]. However, virus clearance was associated with acute respiratory disease which was often lethal when large numbers of cells from a virus-specific T cell line or clone were transferred. Although this demonstration indicates that virus specific cytotoxic T cells can be of benefit in virus clearance there is some risk associated with their deliberate induction by vaccination. If it does not prove possible to dissociate the beneficial and detrimental elements of a cytotoxic T cell response it may be prudent to choose a vaccine which relies for its protective effect upon the generation of protective antibodies.

**Hypersensitivitiy**

The exacerbation of natural infection by prior vaccination of human infants with a formalin inactivated vaccine clearly cannot be repeated for further study. Attempts have been made, therefore, to reproduce the phenomenon in animals. An animal model would be an invaluable tool in the development of alternative vaccine strategies which must be assessed for disease potentiation before clinical trials. Cotton rats immunised with a formalin-inactivated vaccine developed a neutrophillic, alveolar infiltrate on challenge with live virus [94,99]. However, similar, if less intense, inflammatory reactions occurred on RS virus challenge of control animals immunised with formalised, uninfected tissue culture or a formalin killed parainfluenzavirus type 3 vaccine [77,95]. This suggests that responses to cell or serum antigens which are common to both vaccine and challenge inocula are, in part, responsible for the observed post-challenge lung damage in this model. The results of candidate vaccine assessment in this system must therefore be interpreted with care as changes in contamination of the immunising inoculum with cell culture antigens may affect potentiation of disease on challenge independently of the levels of any sensitisation to viral proteins.

Cotton rats receiving formalin-inactivated virus vaccine or live virus generated similar levels of antibody detectable by ELISA against affinity-purified F and G viral glycoproteins. However, the neutralising capacity of the antibody produced in response to formalin-killed virus was reduced [99]. It was suggested, therefore, that an Arthus-type reaction triggered by immune complexes formed between replicating virus and non-neutralising antibody might contribute to the enhanced lung damage seen after challenge.

These animal studies stimulated a re-examination of stored sera collected from infants and children who received formalin-inactivated RS virus vaccine [73]. In comparison with naturally infected infants of similar age and with older vaccinees, vaccinated infants developed similar levels of ELISA antibody to the F protein but lower levels of anti-G antibodies. After natural infection, vaccinees produced unexpectedly poor immune responses to the virus which might have permitted a more severe infection. The most striking observation, however, was that, as in the animal model, the vaccine induced antibody with a lower ratio of neutralising titre to ELISA titre and low levels of fusion inhibiting activity [74].

Subsequent work, however, does not favour a major role for non-neutralising antibody in vaccine-induced hypersensitivity. Firstly, passive administration of non-neutralising monoclonal antibodies to animals has not resulted in potentiation of disease on subsequent challenge although these investigations have not been comprehensive in exploring the effects of epitope specificity or im-
munoglobulin sub-class [110]. Further, cotton rats immunised with purified viral glycoproteins produced antibody similar to that induced by formalin-killed vaccine, with high ELISA and low neutralising titres, but the animals were protected from challenge with little apparent potentiation of disease [77].

An alternative and most instructive model has been provided following the availability of vaccinia virus recombinants expressing individual RS virus proteins. A peribronchial and perivascular, predominantly lymphocytic infiltrate was observed in the lungs of mice challenged with RS virus post-immunisation with vaccinia virus recombinants carrying RS virus F, G or N genes [109]. Lesion scores were higher in vaccinated than in non-vaccinated mice although RS virus could only be isolated from the lungs of the latter. A further vaccinia recombinant expressing the RS virus non-structural 1B protein did not pre-dispose mice to lung damage suggesting that neither the vaccinia virus nor cell culture antigens in the recombinant vaccine were responsible for sensitisation of the mice.

Vaccinia virus recombinants expressing RS virus proteins induce both humoral and cell-mediated immunity [6,87,88]. Passive transfer of anti-RS virus monoclonal or polyclonal antibody to mice, cotton rats or primates prior to infection has not resulted in potentiation of disease on subsequent challenge [38,97,110,120]. However, as discussed above, a large proportion of X-irradiated mice persistently infected with the virus developed respiratory distress, fatal in several cases, after receiving passive transfer of virus specific cytotoxic T cells [15]. Histological examination of the lungs of the infected recipient mice revealed peribroncholar infiltration of lymphocytes, monocytes and polymorphonuclear leukocytes with areas of alveolar consolidation and haemorrhage. It is tempting to equate this phenomenon with the inflammatory changes observed both in inactivated vaccine recipients and vaccinia virus recombinant recipients after challenge. However, several questions must be answered before this view can be accepted. Although formalin-inactivated vaccine induced a T cell response in infant vaccinees as measured by a lymphocyte transformation assay, [51], no attempts have been made to demonstrate that virus inactivated in this way can induce cytotoxic T lymphocyte responses. Further, although vaccinia virus recombinants carrying the RS virus F and M2 protein genes have proved capable of inducing RS virus specific cytotoxic T cell responses in mice [91], the G, N and 1B protein recombinants have not been tested. The G protein was not recognised by mouse cytotoxic T cells generated by virus infection [6]. Although both inactivated vaccine and vaccinia recombinants would be expected to stimulate class 2 restricted T cells, the possibility that cells of this type might potentiate disease has not been investigated.

Alternative mechanisms have been suggested for the potentiation of disease following vaccination against RS virus. In vitro, antibody mediated, Fc receptor-dependent enhancement of RS virus infection of macrophage cell lines has been reported [30,56]. It has been speculated that this mechanism may explain not only potentiation of disease post-vaccination but why infants with declining levels of transplacentally acquired antibody are so uniquely vulnerable to severe lower respiratory tract disease when infected with RS virus. Little evidence exists to support an in vivo role for this mechanism, however, as no correlation between presence of passive antibody and severity of infection or disease has been observed in animals or human infants [86,110,115].

Antigenic variation

Analysis of RS virus isolates, initially by neutralisation tests with animal and human convalescent sera and latterly with monoclonal antibodies, has revealed two distinct subgroups, A and B [2,28,69,123]. Infants infected with subgroup A strains secrete virus for longer and are more likely to be severely ill than those infected with subgroup B strains [112]. Groups in the Americas, U.K., Sweden and Japan have determined the incidence of isolation of viruses of the two subgroups from epidemics over the past 15 years [1,29,41,68,71,112,119]. Numbers of strains characterised are generally small but serve to show that both subgroups can be isolated from virtually
all epidemics and that the relative proportions of the two subgroups isolated varies widely. No global pattern of variation has emerged. Thus, despite minor differences in virulence, both viruses are responsible for significant morbidity and a successful vaccine must protect against both.

At the molecular level, variation between the two subgroups is most pronounced in the viral attachment glycoprotein, G. The amino acid sequence correspondence between subgroups for the G glycoprotein is 53% compared to 94% between strains within a subgroup [46,92]. The sequence of the fusion or F glycoprotein is approximately 90% conserved as are all the other structural and non-structural proteins of the virus with the exception of the SH protein, a small glycoprotein of unknown function thought to be associated with the viral membrane, which is only 76% conserved [19,48].

To what degree immunity is cross-reactive between subgroups is not yet clear. The lungs of cotton rats were well protected against challenge with the heterologous subgroup although virus was recovered after challenge from the upper respiratory tract indicating that cross-protection was not complete [98]. Convalescent antisera taken following primary infection of cotton rats [47] or human infants [43] react similarly with the F glycoprotein of both virus subgroups in ELISA assays. However, reactivity is markedly higher to the G glycoprotein of the homologous rather than the heterologous subgroup and these sera also neutralise virus of the homologous subgroup to a higher titre. Studies of mice infected with vaccinia virus recombinants expressing RS virus F or G glycoprotein genes confirm that the F protein is the major element in the induction of cross-protection in the lung [109]. It is possible that responses to the attachment glycoprotein are more significant in the upper respiratory tract.

If antigenic heterogeneity contributes to the facility with which RS virus is able to reinfect in human infants, the virus isolated on second infections might be expected to be of a different subgroup than that causing the first. Pothier et al. [96] have reported two reinfections, separated by 2 and 4 weeks. In both, second infections were with heterologous subgroup viruses. In the largest study of this type to date, involving 13 children with repeat infections, reinfection with a heterologous virus was more frequent than predicted given the relative prevalence of the two subgroups in the population [70].

One major, as yet unresolved, question is whether the two subgroups are currently undergoing antigenic change under pressure of immunity in the population and whether this change is directional. Both subgroups are antigenically heterogeneous and epitopic differences between strains of the same subgroup have been detected on the G glycoprotein [2,84,106], the nucleoprotein [2] and the 22 kDa M2 protein [84]. Currently circulating subgroup A strains may lack epitopes on the G and M2 proteins present on prototype strains [42,68]. The recent sequencing of a number of G glycoprotein genes from subgroup B strains isolated over 29 years has revealed no sequential or cumulative pattern of mutation. Nonetheless, a higher number of base changes resulted in amino acid changes than expected from chance alone suggesting some form of pressure selecting for phenotypic change (Dr. G. Wertz – personal communication).

Second generation candidate vaccines

The failure to develop suitable attenuated virus vaccine strains and the perceived danger of alterations in viral antigens by inactivation techniques has stimulated interest in the development of subunit vaccines. Passive immunisation of mice with murine monoclonal antibodies indicated that antibodies specific for both the fusion (F) and attachment (G) viral glycoprotein were capable of conferring protection. Immunisation of mice [102] or cotton rats [77] with immunoaffinity purified surface glycoproteins but not internal virion proteins induced neutralising antibody responses. On challenge virus replication was reduced in the lungs although not in the upper respiratory tract. Such preparations clearly have potential as vaccines and toxicity studies have been carried out with immunoaffinity purified F protein adsorbed to alum in adult volunteers and further studies in
children are in progress [44]. As antigenic variation is largely restricted to the G glycoprotein, and neutralisation epitopes on the F glycoprotein are conserved [26], purified F glycoprotein would be expected to induce immunity effective against all virus strains. Whether these preparations will potentiate disease in human infants on natural infection remains a difficult question. Cotton rats immunised with purified glycoproteins exhibited lower levels of pulmonary pathology on challenge with live RS virus than those receiving formalin inactivated virus [77]. However, this result might be expected as the contaminating cell culture antigen content of the subunit vaccine is reduced (see above). No studies of the efficacy of such a vaccine in the presence of residual maternal antibody have been reported. The introduction of RS virus genes into vaccinia virus [4,87], and more recently into SV40 and adenovirus 5 [18], offers novel possibilities for vaccination. Recombinant vaccinia viruses carrying the F or G glycoprotein genes inoculated intradermally into mice, cotton rats and primates induce serum neutralising antibody [88,109]. The degree of protection afforded, however, varies with the species. Although vaccinia recombinants containing the F glycoprotein gene gave essentially complete protection of the lower respiratory tract against both homologous and heterologous virus subgroups in rodents and owl monkeys, protection of the upper respiratory tract was incomplete at best. In chimpanzees, which are closer to human beings in their natural susceptibility to the human virus, intradermal inoculation of vaccinia virus recombinants carrying RS virus F and G genes produced disappointing serum antibody responses and poor protection against challenge [17]. In mice administration of vaccinia recombinants via the respiratory tract induced high titres of antibody in the lung [109] although infection by this route was associated with lung damage and dissemination of the recombinant virus to the brain [114]. A recombinant adenovirus type 5 carrying the RS virus F protein was protective in cotton rats when administered by the respiratory route [18] and experiments with chimpanzees are underway.

In view of the failure of live attenuated vaccines administered by the intramuscular route, it is clearly important to establish the immunogenicity of such candidate vaccines in the presence of high titre passively acquired antibody. Murphy et al. [74] have attempted to investigate this problem for vaccinia virus recombinants in the cotton rat model. Passively administered hyperimmune serum reduced RS virus-specific IgG responses to intradermally administered recombinant vaccinia virus carrying the F and G genes and favoured the production of antibody with low neutralising activity. The immunosuppressive effect was much reduced, however, if the vaccinia virus recombinants were administered intranasally. Complete protection of the lung and significant protection of the upper respiratory tract was achieved indicating a possible way forward for future vaccines [76]. The possibility that recombinant vaccinia viruses carrying viral glycoprotein genes may potentiate disease on infection with RS virus has already been discussed.

Towards a third generation

The continued suspicion that whole virus proteins may sensitise the host to severe disease on natural exposure to the virus has prompted an even finer analysis of viral antigenicity down to the epitope level. Separation of B cell epitopes which engender protective, neutralising antibody responses from those which induce non-functional antibody and T cell epitopes which stimulate helper responses from those which induce potentially damaging cytotoxic T cells may pave the way to a third generation of vaccines containing only selected B cell and T cell epitopes presented either as peptides or as live, recombinant, viral or bacterial vaccines.

B cell epitope mapping has begun with the characterisation of neutralising monoclonal antibodies binding to the viral glycoproteins. Competitive binding and reaction with monoclonal antibody resistant mutants and natural variants isolated in the field consistently reveal four major sites on the viral fusion protein each comprised of a number of distinct epitopes [10,26,116,121]. Antibodies binding to only three sites are capable of neutralising the virus, and one of these which
induces antibodies of higher neutralising titre than the other two, has been designated the major neutralisation site. Antibodies to this site and one other neutralisation site also inhibit syncytium formation although some antibodies to the third site may also exhibit this function. The major neutralisation site is well-conserved; antigenic differences both between and within subgroups occurring at the minor neutralisation sites.

Sequencing of escape mutants suggests that F protein amino acids 262 and 268 are crucial to the integrity of the site [60]. Liu et al. [unpublished results] have demonstrated binding of a neutralising antibody to an F protein fragment expressed in *E. coli* which includes this region of the protein. An escape mutant from a further neutralising, fusion inhibiting monoclonal antibody, which has not yet been fitted into the competitive binding map, has an amino acid change at 429 and synthetic peptides corresponding to this region have been shown to bind to the antibody (Dr. G. Taylor, personal communication).

Neutralising monoclonal antibodies have been shown to bind to peptides from several regions of the F protein generated by proteolytic cleavage techniques or synthesis [12,103,117]. Trudel et al. [117] demonstrated binding to peptides containing F protein amino acids 221 to 232. However, peptides including this region coupled to carrier proteins or RS virus T cell epitopes, failed to induce antibody reacting with virus in ELISAs and was not protective in mice despite a report of low levels of neutralising activity in mouse sera [58,118]. Bourgeois et al. [12], using a similar approach, identified a peptide comprising F protein amino acids 205 to 225 which induced antibody in rabbits recognising virus-infected cells in ELISAs and capable of neutralising the virus. It is not clear how this site relates to the major neutralisation epitope identified by competitive binding.

Topographical maps of the G glycoprotein present a very different picture. Competitive binding studies indicate a large number of independent epitopes with varying degrees of overlap. The majority of epitopes on this glycoprotein are subgroup specific. However, several conserved epitopes have been described and Walsh et al. [122] found such antibodies could neutralise both A and B viruses and were protective in animal studies. Norrby et al. [85] demonstrated that polyclonal antibodies bind to peptides from four regions of the protein but only one peptide 174-188 was recognised by a panel of monoclonal antibodies. None of these monoclonal antibodies were either neutralising or cross-reactive. Mutants resistant to a type-specific, neutralising, monoclonal antibody have been generated and sequencing studies should pin-point amino acids contributing to antibody binding. However, these mutants retained reactivity with cross-reactive antibody epitopes [26].

The mapping of T helper cell epitopes is also underway. In mice, Openshaw et al. [90] found T helper cell epitopes on the fusion glycoprotein and nucleoprotein, but not the G glycoprotein, SH or 1B proteins expressed from vaccinia recombinants. However, using synthetic peptides, Nicholas et al. [82,83] have demonstrated two distinct T helper cell epitopes within a 16 amino acid sequence from the SH protein. Together these were capable of stimulating T cells from mice with several major histocompatibility complex haplotypes. Several hydrophilic peptides from the F protein have also been reported to stimulate IL-2 production by splenocytes from mice primed with RS virus [118].

The incorporation of the SH glycoprotein T cell epitope into a chimaeric peptide with putative epitopes from the G or F glycoproteins successfully enhanced the antibody response of mice to these epitopes. Clearly this approach will be of value if peptides encoding B cell epitopes capable of inducing neutralising antibody formation can be identified.

Similar studies are underway with human beings [1]. Whilst some individuals responded to T helper cell epitopes on the attachment glycoprotein, the phosphoprotein and the 22K M2 protein, all immune subjects tested reacted most strongly to the F glycoprotein. This gives some ground for hope that a relatively small number of T helper cell epitopes from the fusion protein may be able to stimulate responses in the majority of the human population.
ACKNOWLEDGEMENT

I would like to thank Dr. M.J. Carter for his constructive criticism of this manuscript.

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Reference omitted.


[100] Public Health Laboratory Service Communicable Dis-


