
Anti-respiratory syncytial virus monoclonal antibodies show promise in the treatment and prophylaxis of viral disease

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Introduction

Viral respiratory disease is the single most common cause of hospitalization of children in the western world and a significant cause of mortality in the developing world. Respiratory syncytial (RS) virus is one of the major causes of lower respiratory tract disease in children, being responsible for disease in approximately 50% of children hospitalized with bronchiolitis and 25% of children hospitalized with pneumonia [1]. The cost of hospitalizations due to RS virus in the United States alone has been estimated to be $300,000,000 annually. Infections occur as annual winter epidemics, and morbidity and mortality are greatest in infants younger than 6 months of age, premature infants, children with underlying cardiac or pulmonary disease, immunodeficient children and adults and the elderly [2]. Immunity after RS virus infection is incomplete, so that reinfection is common throughout life. However, disease is usually less severe upon reinfection and more likely to be limited to the upper respiratory tract. There is a clear need for an effective vaccine; however RS virus vaccine development has met with difficulties. In particular, a formalin-inactivated RS virus vaccine that was given to infants in the 1960s not only failed to protect against infection, but resulted in
enhanced pulmonary disease when the children became infected [3,4].

RS virus is an enveloped, negative-stranded RNA virus belonging to the genus Pneumovirus in the family Paramyxoviridae (see [3]). The viral genome codes for eight structural and two non-structural proteins. The fusion protein F, the attachment protein G and the SH protein are glycosylated and, together with the 22-kDa protein, are expressed on the cell membrane. The F protein enables fusion of the virus with host cell membranes and the fusion of cell membranes to produce multinucleated giant cells. Although RS virus is monotypic there are antigenic differences among strains of RS virus, and two subgroups (A and B) of the virus exist [6]. It has been estimated that there is about 50% antigenic relatedness in the F protein but only about 5% relatedness in the G protein between the two subgroups. The two subgroups circulate simultaneously in the community, and it has been suggested that the antigenic diversity may be responsible for the occurrence of reinfections [7].

The role of antibody in RS virus infection
Since most severe disease occurs at a time when maternal antibody is present, it has long been thought that antibody may play a harmful role in the pathogenesis of RS virus disease. One explanation for the enhanced pulmonary disease seen in recipients of the formalin-inactivated vaccine was that vaccine-induced serum antibodies, in the absence of a mucosal immune response, may have caused an Arthus reaction in the lungs after RS virus infection. However, there are several lines of evidence that indicate that antibodies protect against RS virus infection and illness. Thus, there is a correlation between levels of maternal antibodies to RS virus and resistance to infection during the first months of life, when the risk of severe disease is greatest [8]; the passive transfer of convalescent serum from RS virus-infected experimental animals (reviewed in [9]), monoclonal antibodies (MAbs) to the F and G glycoproteins of RS virus [10,11] or pooled human immunoglobulin (IVIG) containing high levels of neutralizing antibodies to RS virus [9] can protect experimental animals against RS virus infection. Contrary to the suggestion that antibody to RS virus may be harmful, the passive transfer of antibody to RS virus-infected experimental animals prevented lower respiratory tract disease. Furthermore, sera from cotton rats immunized with formalin-inactivated RS virus that developed exacerbated lung pathology after RS virus infection failed to produce enhanced disease after passive transfer to RS virus-infected cotton rats [12]. Studies with MAbs in mice and human IVIG in cotton rats and owl monkeys also showed that antibody not only protected when given before RS virus infection but could clear an established infection when antibody treatment was delayed until 4 or 5 days after infection [9,13]. These observations that antibody could protect against pulmonary RS virus infection and disease prompted studies into the ability of human IVIG containing high levels of neutralizing antibody to protect against RS virus infections in young children.

Prophylactic and therapeutic effects of human IVIG in children
Studies in cotton rats showed that protective levels of neutralizing antibodies in the circulation, produced by passive transfer of IVIG, were 1:200 to 1:350 [14] and that the microneutralization assay best identified batches of human IVIG that protected animals against RS virus infection [15]. A batch of commercial human IVIG containing high levels of neutralizing antibody to RS virus was given to a group of 17 children hospitalized with RS virus infection [16]. The children, who were given 2 g/kg of the human IVIG, had significant reductions in titres of virus from the upper respiratory tract and a significant improvement in oxygenation 24 h after treatment when compared with a group of 18 children given a placebo.

In a trial to examine the prophylactic effects of human IVIG, a batch was selected that was enriched for neutralizing antibody to RS virus (RSVIG) and given to children at high risk for RS virus disease. The group of children given monthly infusions of 750 mg/kg of RSVIG had significantly fewer lower respiratory tract infections, hospitalizations, days in hospital and days in intensive care than control children [17]. These studies with RSVIG stimulated research into the development of human MAbs specific for RS virus.

Immunoprophylaxis by MAbs to RS virus
One of the major advantages of the use of MAbs to RS virus compared with polyclonal sera is that the higher concentrations of specific antibody that can be achieved with MAbs should decrease the amount of immunoglobulin required for pro-
phylaxis or therapy to doses that can be more easily administered by either the intramuscular or intravenous routes. The F and G proteins of RS virus are the major neutralizing antigens and, as mentioned above, MAbs to the F and G proteins can protect mice and cotton rats against RS virus infection [10,11]. In addition, MAbs to the F and G proteins were also able to clear an established RS virus infection. However, a comparison of the ability of MAbs to clear RS virus from the lungs of persistently infected nude mice showed that a neutralizing MAb to the F protein was much more effective than a neutralizing MAb to the G protein [13]. Furthermore, protective MAbs to the F protein were cross-reactive, whereas the majority of MAbs to the G protein were either RS virus subgroup or strain specific.

An examination of the biological properties of protective MAbs showed that only one out of six protective MAbs to the G protein neutralized RS virus, whereas all the highly protective MAbs to the F protein were neutralizing [13]. However, protection against infection correlated with the ability of the MAbs to inhibit fusion of RS virus-infected cells rather than with neutralization titres [18].

These observations indicate that MAbs to the F protein that neutralize, inhibit fusion and are cross-reactive should be selected for prophylaxis and/or therapy of RS virus infections in man.

Prophylactic and therapeutic effects of a reshaped human MAb (RSHZ19) to RS virus
The complementarity-determining regions from a neutralizing, fusion-inhibiting, murine MAb (Mab 19) [18] that both prevented and cleared RS virus infection in mice were transferred to a human IgGl MAb. After minimal alterations to the framework, consistent with retention of antiviral activity, a reshaped human MAb, RSHZ19, was produced [19]. The dose of RSHZ19, given intraperitoneally, required to completely protect or clear RS virus from the lungs of mice was 5 mg/kg and was the same as that of the original murine MAb [19]. A dose of 2.5 mg/kg of RSHZ19 given to nude mice on day 4 of infection completely eliminated RS virus within 24 h, and virus did not reappear in the lungs, even when RSHZ19 had declined to undetectable levels 2 months later. In contrast, lung virus titres remained high in untreated control nude mice. The EC50 value of RSHZ19 for inhibition of RS virus-infected cell fusion was 6.3 µg/ml, which was similarly to that for the original murine antibody [19]. Initial studies showed that RSHZ19 recognized cells infected with 13 subgroup A and 11 subgroup B isolates of RS virus. Further studies showed that RSHZ19 reacted with RS virus in nasopharyngeal aspirates obtained from infants hospitalized with RS virus infection and five subgroup A and four subgroup B strains were neutralized by RSHZ19 to the same extent (EC50 0.4–3.0 µg/ml) in a microneutralization assay [19a]. RSHZ19 was effective both prophylactically and therapeutically against infection of cotton rats with either subtype A or subtype B RS viruses and significantly reduced the severity of the lung lesions [19a]. A neutralizing titre of 1:32 in cotton rat sera correlated with protection against RS virus infection. This was significantly lower than the levels of neutralizing antibody in the circulation (1:200 to 1:350) of cotton rats passively immunized with human IVIG that were required for protection [14].

A comparison of the prophylactic effects of RSHZ19 with that of a preparation of human serum immunoglobulin (Ig) showed that RSHZ19 was significantly more protective than the human serum Ig [19a]. Thus, a dose of 10 mg/kg RSHZ19 produced a neutralizing titre of 1:78 in the circulation of cotton rats and completely protected against RS virus infection, whereas a dose of 1000 mg/kg human serum Ig was required to produce similar levels of neutralizing antibody, but only reduced virus titre in the lungs by 16-fold.

Thus, RSHZ19 is effective against a wide range of clinical isolates of RS virus and doses of about 5 mg/kg can prevent and clear RS virus infection in mice and cotton rats. High levels of expression of the antibody have been achieved in a Chinese hamster ovary cell line. These findings have encouraged the further clinical development of RSHZ19 for prophylaxis and therapy of RS virus infection in children.

Safety and pharmacokinetics of RSHZ19
A preclinical evaluation of RSHZ19 showed that there was no binding to human tissues by immunohistology, and no evidence of toxicity in adult cynomolgus macaques or infant baboons after single intravenous doses of up to 200 mg/kg, repeat intravenous doses of up to 40 mg/day−1, kg−1 or two intramuscular doses of up to 40 mg/day−1·kg−1. After intravenous administration with 1 mg/kg, the half-life of RSHZ19 was 21–24
days in monkeys [19b]. Absorption of RSHZ19 after intramuscular administration to cynomolgus monkeys was rapid and resulted in levels of systemic exposure similar to that after an intravenous dose. After a single intravenous administration of 200 mg/kg or intramuscular administration of 40 mg/kg to macaques, one out of three animals in each group developed anti-RSHZ19 antibodies, which resulted in the rapid elimination of RSHZ19 from the plasma. The monkeys' immune response was directed primarily towards the human framework region of RSHZ19. After administration of a second intramuscular dose to macaques, no additional animals developed anti-RSHZ19 antibodies.

Single intravenous doses of RSHZ19 ranging from 0.025 to 10 mg/kg were found to be safe and well tolerated in healthy adult male volunteers. RSHZ19 had a half-life of approximately 23 days, similar to that of native IgG, and no volunteers developed anti-RSHZ19 antibodies during 10 weeks of observation [20]. The safety of RSHZ19 in adults bodes well for its safety in infants and children.

**Human monoclonal Fab fragments derived from a combinatorial library**

Although whole IgG, which has a half-life of approximately 23 days, is needed in passive prophylaxis to maintain high levels of protective antibodies in the circulation of individuals at high risk of RS virus infection, it has been suggested that topical administration of Fab fragments may offer advantages over whole IgG for therapy. Thus, large quantities of human antibody Fabs, from combinatorial libraries prepared in filamentous DNA phage, can potentially be produced easily and inexpensively in bacteria. In addition, the use of Fab fragments avoids the potential tissue-damaging effects of immune complex formation and activation of complement. Furthermore, topical administration of antibody reduces the amount required to protect against RS virus infection by about 100 times when compared with the parenteral route [21]. A human monoclonal Fab fragment, derived from a combinatorial library, neutralized RS virus with a high efficiency in tissue culture and was effective against RS virus infection when administered intranasally to mice 3 days after infection [22,23]. However, the therapeutic effect was not sustained, and RS virus reappeared in the lungs 2 days after treatment. This rebound was prevented by daily intranasal administration of the Fab for 3 days. Although Fab fragments may offer some advantages over whole IgG, a rebound of RS virus in the lungs of persistently infected nude mice has not been observed after treatment with RSHZ19 administered either intraperitoneally or intranasally.

**Conclusions**

Despite the clinical and economic importance of RS virus infections, there is no effective vaccine available and the safety and clinical efficacy of the antiviral drug, ribavirin, remain controversial (see [24]). Studies in experimental animals have shown that antibody can protect against RS virus infection and does not contribute to lung pathology. These findings have led to clinical trials of human IVIG preparations containing high levels of neutralizing antibody, which have shown that high doses of antibody can prevent RS virus disease and may be effective therapeutically. MAbs to the F protein, which are fusion inhibiting, are highly effective in both the prevention and treatment of RS virus infection in experimental animals. Although MAbs have significant advantages over polyclonal serum, it may be necessary to use a combination of MAbs, which recognize distinct epitopes on the F protein to prevent the emergence of antibody-escape mutants in antibody-treated individuals. The development of a reshaped human MAb, RSHZ19, which is highly effective against RS virus infection in small laboratory animals, in much lower doses than human serum Ig, offers the greatest promise for the prevention and treatment of RS virus infections in children.
Antibody fragments for controlled delivery of therapeutic agents

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Introduction
Monoclonal antibodies were for a long time hailed as magic bullets that would bring dramatic improvements to the management of a wide range of disease conditions, such as cancer, viral infection, septic shock, transplant rejection, rheumatoid arthritis, thrombosis, etc. Unfortunately, their success has remained limited to in vitro diagnostic and immunoassay applications, and they have failed to live up to these initial, very high expectations. The slow rate of progress can be attributed to a number of reasons. However, rapid and exciting developments during the past decade in the antibody engineering field may finally have turned the table in favour of antibody-based reagents. In future we may finally have the tools to harness the tremendous potential of these versatile molecules not only in the medical but probably also in the industrial sector.

Therapeutic antibodies: the issues
A number of factors that are directly related to the biochemical and physical properties of antibodies have seriously hampered progress towards their successful application:

1. The occurrence of human anti-mouse antibody (HAMA) response to injected murine antibodies. Further administration of the same antibody results in rapid clearance, thus precluding further treatment. In some cases anaphylactic shock can also occur [1]. Human antibodies should be more suitable.

2. Lack of sufficient specificity. The majority of cancer markers are not exclusively found on tumours. They are often found in healthy tissue as well, albeit in smaller amounts, thus limiting the opportunity to an operational ‘window’ of specificity.