Vaccine xxx (2016) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Animal models of respiratory syncytial virus infection

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ARTICLE INFO

Article history:
Available online xxxx

Keywords:
Respiratory syncytial virus
Animal models

ABSTRACT

Human respiratory syncytial virus (hRSV) is a major cause of respiratory disease and hospitalisation of infants, worldwide, and is also responsible for significant morbidity in adults and excess deaths in the elderly. There is no licensed hRSV vaccine or effective therapeutic agent. However, there are a growing number of hRSV vaccine candidates that have been developed targeting different populations at risk of hRSV infection. Animal models of hRSV play an important role in the preclinical testing of hRSV vaccine candidates and although many have shown efficacy in preclinical studies, few have progressed to clinical trials or they have had only limited success. This is, at least in part, due to the lack of animal models that fully recapitulate the pathogenesis of hRSV infection in humans. This review summarises the strengths and limitations of animal models of hRSV, which include those in which hRSV is used to infect non-human mammalian hosts, and those in which non-human pneumoviruses, such as bovine (b)RSV and pneumonia virus of mice (PVM) are studied in their natural host.

Apart from chimpanzees, other non-human primates (NHP) are only semi-permissive for hRSV replication and experimental infection with large doses of virus result in little or no clinical signs of disease, and generally only mild pulmonary pathology. Other animal models such as cotton rats, mice, ferrets, guinea pigs, hamsters, chinchillas, and neonatal lambs are also only semi-permissive for hRSV. Nevertheless, mice and cotton rats have been of value in the development of monoclonal antibody prophylaxis for infants at high risk of severe hRSV infection and have provided insights into mechanisms of immunity to and pathogenesis of hRSV. However, the extent to which they predict hRSV vaccine efficacy and safety is unclear and several hRSV vaccine candidates that are completely protective in rodent models are poorly effective in chimpanzees and other NHP, such as African Green monkeys. Furthermore, interpretation of findings from many rodent and NHP models of vaccine-enhanced hRSV disease has been confounded by sensitisation to non-viral antigens present in the vaccine and challenge virus.

Studies of non-human pneumoviruses in their native hosts are more likely to reflect the pathogenesis of natural hRSV infection, and experimental infection of calves with bRSV and of mice with PVM result in clinical disease and extensive pulmonary pathology. These animal models have not only been of value in studies on mechanisms of immunity to and the pathogenesis of pneumovirus infections but have also been used to evaluate hRSV vaccine concepts. Furthermore, the similarities between the epidemiology of bRSV in calves and hRSV in infants and the high level of genetic and antigenic similarity between bRSV and hRSV, make the calf model of bRSV infection a relevant model for preclinical evaluation of hRSV vaccine candidates which contain proteins that are conserved between hRSV and bRSV.

1. Introduction

Human respiratory syncytial virus (hRSV) is the commonest cause of lower respiratory tract infection (LRTI) in children, worldwide, causing disease in an estimated 34 million children, >3 million hospitalizations, and 66,000–199,000 deaths in children under 5 years, each year [1]. Most children are infected during the first year of life, and all have been infected by their second year. Although there is limited viral antigenic variation, the duration of immunity induced by hRSV is short-lived and recurrent infections occur throughout life [2]. The peak incidence of severe disease is in infants 2–7 months of age. However, hRSV also contributes to excess mortality in the elderly [3,4] and severe hRSV disease occurs in immunosuppressed individuals of any age [5]. The economic impact of hRSV disease in adults is estimated to be greater than that of influenza in relation to numbers of days lost from work [6,7]. There is no effective anti-viral therapy or licensed hRSV vaccine. However, monoclonal antibody (mAb) prophylaxis is effective in reducing hRSV hospitalisations by 55% in infants at increased risk of severe disease [8].

Hurdles to vaccine development include the need to vaccinate at an early age when maternal antibodies are present, the failure

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http://dx.doi.org/10.1016/j.vaccine.2016.11.054
0264-410X/

of natural infection to prevent reinfection, a history of vaccine-enhanced disease in young children given a formalin-inactivated (FI) hRSV vaccine [9], and the lack of animal models that fully reproduce the pathogenesis of hRSV infection in man. Since hRSV infection does not prime for enhanced disease, live attenuated vaccine candidates are considered safe, although their protective efficacy may not be very durable. Development of other vaccines for paediatric use is hampered by the difficulty of reliably demonstrating their safety and efficacy in preclinical studies.

2. HRSV infection in man

HRSV is mainly transmitted by large particle aerosol or direct contact. Initial viral replication occurs in the nasopharynx with an incubation period of 4–5 days, and can be followed by spread to the LRT. Disease severity ranges from that of a mild common cold to bronchiolitis, with airway obstruction and hypoxia in infants, wheezing, and pneumonia [4]. Apnea is seen in 1–24% of hRSV-infected infants [10], and severe hRSV bronchiolitis is associated with subsequent episodes of wheezing which can persist until ~11 years of age [11].

HRSV replicates primarily in ciliated airway epithelial cells and type I and type II alveolar pneumocytes (Fig. 1A) [12]. Histopathological changes of fatal cases of hRSV infection are characterised by peribronchiolar and perivascular mononuclear cell accumulations, interstitial pneumonia, bronchiolar epithelial cell necrosis, and occlusion of the bronchiolar lumen by desquamated epithelial cells, macrophages, neutrophils, fibrin, and mucin, resulting in airway obstruction (Fig. 1B) [12]. Cells in the peribronchial areas of the lung of a child with hRSV who died from an unrelated cause, consisted of monocytes, $CD3^+CD4^-CD8^-$ T cells and $CD3^+CD8^+$ [12]. However, $CD4^+$ and $CD8^+$ lymphocytes were rare in lungs of fatal hRSV LRTI [13]. Neutrophils predominate in bronchoalveolar lavage (BAL) from infants with hRSV bronchiolitis [14,15], and respiratory secretions contain high levels of proinflammatory cytokines (e.g. tumour necrosis factor-α (TNFα), interleukin-6 (IL-6), IL-1α), CXC/CC chemokines (e.g. IL-8, MIP-1α, MCP-1 and RANTES) [16], and interferon (IFN)γ, IL-4, IL-5, IL-10, IL-9 and IL-17 [17,18]. There is an association between severity of hRSV LRTI and polymorphisms in a number of host response genes (e.g. IL-8 promoter, and CC chemokine receptor 5 (CCR5), which binds Mip-1α and RANTES) [19,20], suggesting that a robust inflammatory response contributes to hRSV pathogenesis. However, intrinsic properties of different virus strains may also contribute to variations in disease severity [21].

3. Animal models of RSV

Animal models of hRSV include those in which hRSV is used to infect non-human mammalian hosts, and those in which non-human pneumoviruses are studied in their natural host. The main non-human mammalian hosts that have been used are non-human primates, cotton rats, mice, and lambs. Apart from chimpanzees, these animals are semi-permissive for hRSV replication and experimental infection with large doses of virus result in little or no clinical signs of disease. In contrast, natural hosts of non-human pneumoviruses, such as bovine (b)RSV and pneumonia virus of mice (PVM), are fully permissive for virus replication and experimental infection results in disease.

3.1. Non-human primates

3.1.1. Chimpanzees

HRSV was first isolated from a chimpanzee in a captive colony of 20 animals, 14 of which had a respiratory illness characterised by coughing, sneezing, and a mucopurulent nasal discharge [22]. The virus, which was originally termed chimpanzee coryza agent (CCA), caused a similar upper respiratory tract (URT) disease in 2 out of 3 chimpanzees after intranasal (IN) inoculation with 10^4 $TCID_{50}$ of tissue culture-passaged material. The animal that did not develop disease was the only one that had pre-existing hRSV-specific serum antibodies. The virus was transmitted not only between chimpanzees but also from chimpanzees to a laboratory worker.

Chimpanzees experimentally infected with $\sim 10^4$ pfu hRSV shed large quantities of virus (10^5 and 10^6 pfu/ml of nasopharyngeal swab sample and tracheal lavage, respectively), for 6–10 days, which is comparable to that seen in children (Table 1) [23,24]. Although LRTI has not been seen in experimentally infected chimpanzees, fatal cases of hRSV-associated bronchopneumonia, some of which also involved *Streptococcus pneumoniae*, have been reported in captive chimpanzees in zoos and in habituated chimpanzees in a research station in Côte d'Ivoire [25–28]. HRSV antigen can be detected in the lungs and histopathological changes of extensive purulent bronchopneumonia and interstitial pneumonia with occasional syncytial cells resemble those in human infants (Fig. 1E) [25]. *S. pneumoniae* has been shown to enhance hRSV infection in differentiated human airway epithelial cells, *in vitro*, and in cotton rats [29].

Chimpanzees have been used to evaluate the virulence and protective efficacy of live, attenuated hRSV vaccine candidates [24,30,31]. The replication and genetic stability of the mutant viruses in chimpanzees parallels that in sero-negative children, and the mutants induced protection against subsequent wild-type hRSV challenge in chimpanzees [24,32–34]. However, chimpanzees vaccinated with recombinant vaccinia viruses (rVV) expressing the hRSV surface glycoproteins F and G developed only low levels of neutralising antibodies, and were poorly protected against hRSV challenge [24,35]. These findings contrast with the almost complete protection induced in mice, cotton rats and owl monkeys by the same rVV [24,35].

3.1.2. African green monkeys

African green monkeys (AGMs), which are semi-permissive for hRSV replication (Table 1), have been used in a number of studies to evaluate hRSV vaccine candidates [36–41]. Clinical signs of disease are uncommon following hRSV infection, and AGMs develop only mild histopathological changes in the lung [38,42,43], The proportion of neutrophils in BAL of monkeys infected with the Memphis M37 (M37) strain of hRSV increased only to 9%, [40], whereas neutrophils are the predominant cell in BAL from hRSV-infected children [14].

Vaccine-enhanced pulmonary pathology has been demonstrated following hRSV challenge of AGMs vaccinated with FI-hRSV [42]. Although FI-hRSV induced partial protection against hRSV replication, animals developed severe peribronchiolar and parenchymal inflammation. Enhanced histopathological changes were also induced in hRSV-infected AGMs that had been vaccinated with FI-herpes simplex virus (FI-HSV), although they were less severe than those in FI-hRSV animals. These findings suggest that an immune response to cell culture components present in the vaccine and the hRSV challenge may have contributed to the enhanced pathology seen in FI-hRSV vaccinated animals.

A comparison of the protective efficacy of a number of different non-replicating hRSV vaccines, all of which had been shown to induce complete protection against hRSV in mice and/or cotton rats, demonstrated varying efficacy in AGMs [40]. The vaccines included purified hRSV F and G proteins in different adjuvants, replication incompetent recombinant vesicular stomatitis virus expressing F or G, replication incompetent recombinant adenovirus (rAdV) expressing F or G, and DNA plasmids encoding F or

Please cite this article in press as: Taylor G. Animal models of respiratory syncytial virus infection. Vaccine (2016), http://dx.doi.org/10.1016/j.vaccine.2016.11.054