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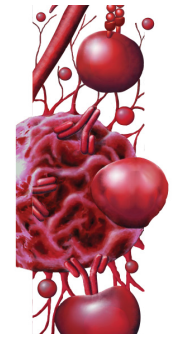
Immunotherapy



Small hydrophobic protein of respiratory syncytial virus as a novel vaccine antigen

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"Possibly the most intriguing features of human respiratory syncytial virus are its ability to infect young infants in the presence of maternal neutralizing antibodies and its ability to reinfect throughout life."

Keywords: human respiratory syncytial virus • small hydrophobic protein • vaccine

Human respiratory syncytial virus (HRSV) is the leading cause of lower respiratory tract infections and associated hospitalizations of infants worldwide [1]. HRSV infects the epithelium of the lung and actively initiates the shedding of infected cells without inducing major cytopathology [2]. When these infected cells are not efficiently cleared by macrophages or T cells, they form clumps that can obstruct the very narrow airways of young infants [3]. Possibly the most intriguing features of HRSV are its ability to infect young infants in the presence of maternal neutralizing antibodies and its ability to reinfect throughout life [4,5]. As such HRSV is also associated with excess morbidity and mortality in elderly people [6].

Why is it that the natural immune responses poorly protect against reinfection with HRSV? First, HRSV evades innate immune responses [7]. In addition, most HRSV infections are restricted to the upper respiratory tract, a site that is poorly accessed by circulating, potentially virus-neutralizing IgG. There is some evidence to suggest that HRSV-neutralizing antibodies inversely correlate with severity of HRSV disease [8–10]. In line with this, prophylactic use of a monoclonal HRSV-neutralizing antibody reduces HRSV-associated hospitalizations of high-risk infants by approximately 50% [11]. However, epidemiological and controlled HRSV challenge studies showed that infants and even healthy adults with high levels of serum antibodies directed against the neutralizing HRSV antigens F and G, remained susceptible to HRSV infection [8,9].

With these observations and the fact that very young infants are an important target group, it is not surprising that decades of HRSV research has not yet resulted in an effective vaccine. As HRSV neutralizing antibodies can provide a degree of protection, most subunit vaccine developments aim at inducing such antibodies. HRSV F and G are the major surface proteins and are known to display neutralizing epitopes [12]. Recently, important progress has been made in developing RSV F and G subunit vaccines, including the breakthrough of the production of recombinant HRSV F protein that is stabilized in its prefusion conformation [13]. Compared to postfusion F, immunization with prefusion F induces higher HRSV-neutralizing antibodies in mice and in nonhuman primates. In addition, antibodies directed against the conserved cysteine noose of the HRSV G proteins, that contain a CX3C motif, were shown to inhibit protein G-CX3CR interaction and reduce HRSV replication and disease [14]. Taking into account the partial immunity evoked by recurrent natural infections, it remains to be seen whether HRSV subunit vaccines solely based on the F and/or G proteins will be able to provide strong long-living immunity.

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Our studies have explored an alternative antibody-accessible HRSV antigen; the ectodomain of the small hydrophobic protein (SH). This membrane protein comprises 64 (HRSV subgroup A) or 65 (HRSV subgroup B) amino acids (AA) and is expressed by all members of the *Pneumovirinae* subfamiliy of the *Paramyxoviridae* family, including Bovine RSV, Ovine RSV and Human Metapneumovirus. In infected cells SH accumulates at the golgi, endoplasmic reticulum and plasma membrane but is hard to detect in HRSV virions [15]. A minor fraction of SH proteins is modified by glycosylation or expressed as a truncated variant [16]. SH forms pentameric cation-selective ion channels and has been reported to activate the NLPR3 inflammasome and to inhibit apoptosis and TNF signaling [17–19]. Evidence suggests that the protein is dispensable for *in vitro* replication of HRSV but HRSV lacking SH are attenuated *in vivo* [20].

The short (HRSV A: 23 AA; HRSV B: 24 AA) SH ectodomain (SHe) appears to be weakly immunogenic upon natural infections. Following HRSV infection, only about half of the children developed a rise in SHe-specific serum IgG [21]. Likewise, SHe-specific IgG levels are very low in convalescent sera from HRSV infected BALB/c mice, cotton rats and in human reference sera [22]. Therefore, we made SHe immunogenic by conjugating synthetic SHe peptide to keyhole limpet hemocyanin. Immunization of BALB/c mice and cotton rats with this conjugate induced high levels of SHe-specific IgG and this immune response was associated with reduced pulmonary HRSV replication and protection against morbidity. However, SHe immune sera, which confers protection against HRSV challenge to naive mice, lack HRSV-neutralizing activity in cell line cultures. We speculate that the low abundance of SH on virions and potential shielding by F and G contribute to this lack of neutralization [15,22]. Furthermore, SH-deficient viruses replicate very efficiently *in vitro* [23]. It is possible that SHe-specific IgG would interfere with HRSV replication in primary human bronchial epithelial cells differentiated on an air-liquid interface. However, we found that in mice lacking Fcγ receptors I and III, SHe immune serum failed to protect, arguing against direct virus neutralization by SHe-specific antibodies *in vivo* [22].

It is well known that next to direct neutralization, antibodies can hamper infections by engaging the complement system or Fcγ receptors, via their Fc tail, to eliminate viral particles or infected cells. For example, the Fc tail of two monoclonal antibodies that recognize the conserved cysteine-noose of the G protein are essential for *in vivo* protection [24,25]. The protection by the non-neutralizing 18A2B2 monoclonal antibody was shown to be dependent on the complement system [25]. In contrast, the observation that both Fab fragments and nanobodies® (the antigen binding domain of cameloid heavy chain-only antibodies) that target the Synagis epitope in F inhibit HRSV replication in mice suggests that these antibodies do not depend on their Fc tail for protection [26,27]. By using *FcγRI/III*$^{-/-}$ mice we could demonstrate the importance of FcγRI and/or FcγRIII in SHe antibody-mediated control of infection. In mice, alveolar macrophages express both FcγRI and FcγRIII whereas NK cells that quickly infiltrate in HRSV-infected lungs only express FcγRIII [28]. Using cell depletion we highlighted an important role for alveolar macrophages, but not NK cells in SHe immune serum-mediated protection. Intriguingly HRSV might have adopted strategies to escape from both neutralizing and FcγR-engaging antibodies. HRSV G is also produced as a secreted form (sG). Recombinant viruses that only express membrane-anchored G proteins and not sG were more sensitive to G-specific neutralizing antibodies *in vitro* but also more sensitive to HRSV or F immune serum-mediated protection *in vivo*. This increased sensitivity could be explained by enhanced Fcγ receptor-mediated uptake of viral particles or infected cells that are opsonized by anti-G antibodies by leukocytes [29]. It would be of interest to investigate whether sG would also affect SHe immune serum-mediated protection of HRSV.

In contrast to F and G, SH is hard to detect on the surface of virions [15,22]. This suggests that in contrast to F- and G-specific antibodies, SHe-specific antibodies control HRSV infection by targeting infected cells rather than HRSV virions. Therefore, we propose that SHe-based vaccination reduced HRSV infection by aiding in the clearance of infected cells. In infants, severe and fatal HRSV infections are caused by airway obstruction by plugs containing HRSV-infected cells shed from the epithelium. These lungs lack HRSV-specific T cells which would normally clear infected cells [3]. Interestingly, airway occlusion by cellular debris also occurs in HRSV-infected New Zealand black mice, which have constitutive deficiencies in macrophage function, and in BALB/c mice with depleted alveolar macrophages [30]. As such SHe-specific antibodies that engage Fcγ receptor-expressing macrophages to remove HRSV-infected epithelial cells might, in addition to reducing HRSV progeny from these infected cells, also avert airway occlusion.

An important question is whether a vaccine such as SHe, that does not induce neutralizing antibodies but aims at elimination of infected cells, could have a clinical benefit? We and others have shown that non-neutralizing antibodies directed against the ectodomain of the influenza matrix protein 2 (M2e), pro-

tect against influenza infections by engaging alveolar macrophages to eliminate infected cells via FcγR I and III [31]. Recently, the therapeutic efficacy of a non-neutralizing human M2e-specific monoclonal antibody (TCN-032) was evaluated in humans by experimental infection. Therapeutic treatment with TCN-032 significantly reduced and shortened viral shedding and symptoms to a similar extent that has been observed for the influenza antivirals; oseltamivir (Tamiflu) and peramivir [32]. These promising results indicate that a vaccine that targets infected cells can have significant clinical benefit upon acute respiratory infections. One can imagine that the possible clinical impact of a therapeutic antibody treatment or a vaccine aiming at controlling viral replication by eliminating infected cells strongly depends on the kinetics of viral replication. In experimentally infected adults, influenza virus shedding peaks at 2 days postinfection, whereas HRSV peaks at 6 days postinfection [33]. In children infected with live attenuated HRSV, viral load peaks at 7–8 days postinfection [34]. This relatively slow kinetics of HRSV infection provides a window of opportunity to control infection by eliminating infected cells. As SHe specific antibodies display a low binding capacity to HRSV virions, those non-neutralizing antibodies would likely not interfere with HRSV-neutralizing antibodies. Hence, a vaccine that combines both SHe with either F or G might be an intriguing vaccination approach.

Financial & competing interests disclosure

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