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Therapeutic targeting of respiratory syncytial virus G-protein

Lawrence M Kauvar^{1,†}, Jennifer L Harcourt², Lia M Haynes², and Ralph A Tripp³

¹ Trellis Bioscience, 2-B Corporate Drive, South San Francisco, CA 94080, USA

² Centers for Disease Control & Prevention, Atlanta, GA, USA

³ University of Georgia, Department of Infectious Diseases, Athens, GA, USA

Abstract

Respiratory syncytial virus (RSV) is a leading cause of pneumonia and bronchiolitis in infants and young children and an important pathogen of the elderly and immune suppressed. The only intervention currently available is a monoclonal antibody against the RSV fusion protein, which has shown utility as a prophylactic for high-risk premature infants, but which has not shown postinfection therapeutic efficacy in the specific RSV-infected populations studied. Thus, for the major susceptible populations, there remains a great need for effective treatment. Recent results support monoclonal antibody targeting of the RSV G-protein for therapeutic use. This objective encompasses a dual mechanism: reduction in the ability of RSV G-protein to distort the host innate immune response, and direct complement-mediated antiviral activity.

Keywords

antibody therapy; innate immunity; prophylaxis; RSV; therapeutics; treatment

Respiratory syncytial virus (RSV) is a leading cause of serious lower respiratory tract illness in infants [1], and mediates significant morbidity in the elderly and those with weakened immune systems [2,3]. RSV infection is typically more prolonged than other respiratory tract infections, such as influenza [1]. This persistence has been shown to correlate with airway hyper-responsiveness (AHR) in a mouse model [4], with both host and viral factors contributing to morbidity [3]. The pathology reflects an airway inflammation syndrome that has features in common with asthma, and children who have had severe RSV disease are at substantially increased risk for the development of childhood wheezing [5,6]. Thus, an effective therapy would ideally ameliorate both acute and long-term RSV-associated symptoms. Premature infants prophylactically treated with a humanized monoclonal antibody (mAb) against RSV fusion (F) protein, specifically palivizumab, have a significantly lower rate of wheezing and associated symptoms compared with controls (13

[†]Author for correspondence: Tel.: +1 650 616 1117, Fax: +1 650 589 4860, lkauvar@trellisbio.com.

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vs 26%) [7]. Unfortunately, neither this nor any other treatment for acute RSV infection has proven to have significant efficacy when given postinfection [8,9], nor has any vaccine candidate been proven effective [10].

Mechanistic investigation of RSV-induced pathology over the past decade has increasingly focused on the RSV attachment (G)-protein, and new support for therapeutic targeting of this glycoprotein continues to emerge. Some pathology associated with RSV infection in animal models has been linked to RSV G-protein-mediated distortions of the immune response that reduce appropriate antiviral cellular responses [3], and molecular dissection of the RSV G-protein has identified a specific CX3C chemokine motif that appears responsible [11]. Importantly, antibodies that block this motif on the RSV G-protein are effective at reducing viral replication and at reducing disease severity in animals [12]. Although the prophylactic efficacy of anti-G mAbs in both mice and cotton rats has been previously established, recent postinfection treatment models have shown that anti-G mAbs are superior to anti-F mAbs at reducing RSV pathogenesis and enhancing viral clearance [13]. These preclinical experiments using mAbs against the RSV G-protein support advancing into clinical testing of a high-affinity mAb derived from B lymphocytes of recovering patients [14].

RSV pathology

Respiratory syncytial virus is a negative-strand RNA virus of the *Paramyxovirus* family, which includes measles and mumps. Although its genome is relatively small, encoding only 11 proteins, RSV is sophisticated in generating the appropriate viral protein ratios to facilitate virus replication [15]. The F and G proteins are the most prominent proteins in the virion capsid and on the surface of cells from which the virus buds. The F-protein is implicated in fusion of the virion to cells, and its deletion leads to drastic reduction in infectivity. By contrast, deletion of the G-protein has a modest effect on replication; however, deletion substantially reduces infectivity *in vivo* [16]. The F-protein is largely conserved across all RSV strains. By contrast, portions of the RSV G-protein are highly variable and heavily glycosylated, which may contribute to evasion of the humoral response [17]. These mucin-like domains may promote percolation through the mucus lining of the respiratory tract, potentially accounting for the decreased infectivity observed *in vivo* in the absence of the RSV G-protein [16]. Detailed study of the RSV G-protein has revealed a small central motif that is highly conserved across all strains [14].

Nearly all children are infected by RSV at least once during the first 2 years of life and approximately half more than once [1]. In severe cases, RSV infection can last several weeks accompanied by copious nasopharyngeal virus shedding that renders the virus highly contagious. A substantial portion of RSV-infected children develop bronchiolitis sufficient to require hospitalization, accounting for over 50% of admissions for lower respiratory tract illness [1]. The etiology of lung pathogenesis associated with RSV infection is complex and multi-factorial, involving both the immune response to infection and the magnitude of viral load. In mice, the overexuberant inflammatory cascade that follows RSV infection may contribute to the development of AHR [18]. The mechanisms that contribute to this pathology are not fully understood, but include mucus secretion and inflammation resulting in bronchoconstriction resembling asthma, a disease characterized by a Th2-biased response [19]. In humans, the cytokine response to RSV was found to be Th2 biased, and even more so in patients with acute bronchiolitis [20]. Cohort studies of fatal RSV cases in Chile have shown that the histo-pathological features of severe RSV infection include airway occlusion with an accumulation of apoptotic cellular debris and increased leukocyte infiltration [21]. In the same study, a similar histopathology was identified following RSV infection in New Zealand Black (NZB) mice, which have constitutive deficiencies in macrophage function.

These findings imply that reduced ability to achieve clearance of infected cells and cellular debris linked to macrophage function contributes to severe disease.

Dysfunctional immune response to RSV

Respiratory syncytial virus infection leads to progressive damage to the lung epithelium and temporal release of a variety of host cell immune modulating substances [3]. In addition, RSV replication in these cells leads to release of its soluble form of G-protein (Gs) [22]. Early evidence that the RSV G-protein is involved in immune modulation came from studies in which mice were exogenously treated with RSV G-protein 6 weeks prior to intranasal infection with wild-type RSV, a protocol that resulted in more severe pulmonary disease [23]. Conversely, mice infected with an engineered virus that does not produce Gs developed a milder disease course compared with wild-type virus, characterized by tenfold lower lung viral load at day 4 postinfection (near the peak for viremia in this model) and a minimal increase in lung infiltrating inflammatory cells at day 7 (the point at which airway inflammation peaks following wild-type virus infection) [24].

One mechanism proposed for the attenuated disease following infection with RSV lacking Gs protein is that the secreted protein acts as a decoy, sequestering antibody that would otherwise be effective at eliminating virus or virus infected cells [25]. Consistent with this hypothesis, it was shown that treatment with an anti-G mAb was more effective in reducing the virus load for the RSV mutant with only surface-expressed G-protein compared with wild-type virus, which also expresses Gs [25]; however, the mutant virus was also more effectively reduced by an anti-F mAb for which the decoy hypothesis is not relevant. That is, RSV Gs apparently modulated the host immune response in a manner that affected the activity of both anti-F and anti-G antibodies.

Modulation of the immune response by RSV is achieved at a variety of levels including effects on innate immunity, the T-cell response to infection, and cell trafficking into the lung, as has been reviewed elsewhere [3,22,26–28]. For example, impaired T-cell stimulation by RSV-infected dendritic cells has recently been reported, although the mechanism remains unclear [29]. One well-studied aspect of innate immune system modulation is the role of the RSV G-protein in suppressing signaling by Toll-like receptor (TLR)4. In cultured epithelial cells, TLR4 is not only stimulated but upregulated by RSV [30] consistent with direct evidence that the RSV F-protein interacts with TLR4 [31]. By contrast, the G-protein reduces TLR4 activity to near baseline levels, even in the presence of a strong stimulus such as lipopolysaccharides, as assayed using a luciferase reporter construct for TLR4 signaling [28]. Downstream of TLR signaling is the suppressor of the cytokine signaling family of proteins that negatively regulate cytokine and chemokine expression, which may be induced through TLR activation [32]. Recent studies have demonstrated that RSV infection of normal human bronchoepithelial cells results in very early TLR activation, and that RSV G-protein modulates suppressor of the cytokine signaling expression to inhibit type I IFN and interferon-stimulated gene (*ISG*)-15 expression [27]. These findings indicate that RSV surface proteins signal through the TLR pathway, suggesting that this may be an important mechanism to reduce type I IFN expression to aid virus replication.

Cell trafficking to the lungs is an important aspect of RSV control and pathology. Fractalkine (CX3CL1) is a chemokine implicated in extra-vascularization of antigen-specific killer T cells and NK cells [33]. The RSV G-protein competes with fractalkine for binding to the fractalkine receptor, CX3CR1, as recently reviewed [3]. CD8⁺ cells expressing CX3CR1 have been shown to be a major component of the cytotoxic response to RSV infection [34]. In this study, CX3CR1⁺ T cells specific for RSV dramatically increased in the lungs of mice

infected with an RSV mutant lacking the G gene, as did the frequency of NK cells; in parallel, Th2-type cytokine expression was reduced. Thus, G-protein is directly involved in at least some of RSV's immunomodulatory effects. Human epidemiology provides correlative support for these results. A mutation in CX3CR1 that reduces affinity for fractalkine was found in 38% of children hospitalized with severe RSV versus 21% in a control group of normal healthy adults ($p = 0.025$) [35]. Similarly, mutations in TLR4 that reduce receptor activity were over-represented in infants hospitalized with RSV for whom there was a 20% frequency versus 5% frequency in infants with mild or no disease ($p = 0.004$) [36]; no such correlation was found for any other TLR [37]. There are other polymorphisms that also correlate with RSV severity [38]; however, these two observations support the hypothesis that G-protein-associated immune modulations play a significant role in clinical severity.

Molecular dissection of RSV G-protein

Although multiple mechanisms contribute to the inadequate immunological response to RSV, the progress in uncovering the role of RSV G-protein has generated interest in defining the portion of the protein that is responsible for its immunological effects. Attention has focused primarily on a CX3C motif located in the cysteine noose central region of the RSV G-protein, and flanking N- and C-terminal residues spanning residues 148–198. This CX3C motif is highly conserved across 37 different virus strains [14]. Furthermore, this region has very low immunogenicity, even as a subunit vaccine [39].

Direct evidence for activity of this motif has been demonstrated by potent suppression of lipopolysaccharide-induced TLR4 activation in cell culture by a 26-mer peptide from this region [31]. This effect was abolished by mutating the cysteines to serines, which presumably caused a major change in peptide conformation. The study further showed that a deletion of the RSV G-protein had a major impact on fractalkine positive cell trafficking. These same effects were also achieved by a cysteine-to-arginine mutation in the CX3C motif [40].

Antibody treatment models

Prophylactic administration of palivizumab, a humanized anti-F mAb, is effective in reducing RSV hospitalizations and disease severity in high-risk infants and young children [41]. Furthermore, premature infants treated with palivizumab have a significantly lower rate of wheezing and associated symptoms compared with controls (13 vs 26%) [7]. A Phase III noninferiority clinical trial for RSV prophylaxis was recently completed [42], comparing palivizumab with motavizumab, a higher-affinity derivative of palivizumab with the same specificity [43]. Motavizumab demonstrated noninferiority compared with palivizumab for the primary end point of RSV hospitalizations. A 26% relative reduction in RSV hospitalizations was observed for motavizumab compared with palivizumab. There was a 50% relative reduction in the rate of RSV-specific outpatient medically attended lower respiratory tract infections, measured as a secondary end point [42,44]. Postinfection treatment with palivizumab was not successful in the specific patient populations in which the studies were conducted [45]. By contrast, motavizumab demonstrated significant antiviral activity at day 1 after treatment in RSV-infected children; however, assessing its full clinical benefit for the treatment of RSV disease will require additional clinical testing [46]. For severe RSV disease, the use of an antiviral treatment together with an anti-inflammatory agent may be appropriate for an effective treatment. Given the known benefits and shortcomings of anti-F mAbs, a shift in focus towards anti-G mAbs is motivated by the improved understanding of the role of RSV G-protein in distorting the host immune response.

Antibodies targeting the central conserved motif of RSV G-protein were shown to be effective for prophylaxis 20 years ago, in both mouse [47] and cotton rat [48]. Sera from elderly subjects vaccinated with an RSV G-protein construct were effective as a prophylactic in nude mice [39]. More recently, higher-affinity mAbs to either RSV G- or F-proteins have been shown to be even more active in these animal models than the first-generation mAb palivizumab [14,43]. Anti-G mAbs that block G-protein binding to CX3CR1 have also shown efficacy in a postinfection treatment mouse model using either murine [13,49], or very-high-affinity human mAbs [14]. In these studies, mice were infected at day 0, intraperitoneally treated with a single injection of mAb at day 3 postinfection, and monitored for viral load at days 5 and 7 either as plaque forming units (pfu) per gram of lung tissue, or by quantitative PCR. Compared with anti-F mAb (i.e., either palivizumab or a murine mAb with the same specificity), the anti-G mAbs were significantly more effective at reducing lower respiratory tract infection. In Figure 1, the increased efficacy of an anti-G mAb compared with anti-F is illustrated for the postinfection treatment mouse model. In the same model, anti-G mAb was also more effective than anti-F mAb at reducing both pulmonary cell inflammation and proinflammatory cytokine production [13]. For these experiments, the antibodies were tested at 5 mg/kg, a dose that is sufficient to abolish viral load in the mouse lungs for all antibodies when given prophylactically [14]. For comparison, palivizumab is administered to humans at 15 mg/kg monthly [41]. With a half-life of approximately 2 weeks, the average dose in humans is close to that used in the mouse model.

Anti-G mAbs appear to have two distinct mechanisms that could facilitate treatment and/or prevention of RSV infection:

- Direct antiviral activity dependent on the intact antibody including the Fc sequence;
- Immunomodulatory activity dependent only on the antigen combining site.

In Figure 2, an anti-G mAb fragment lacking the Fc portion is compared with the corresponding intact IgG. The intact mAb was active as both an antiviral and anti-inflammatory agent, whereas the F(ab')₂ fragment had minimal anti-viral activity but retained efficacy comparable with the intact IgG for reducing the influx of inflammatory cells into the lung [49]. This dual mechanism (i.e., Fc-dependent and independent actions) is consistent with studies in a mouse prophylactic model using chimeric mouse-human anti-G mAbs with and without a glycosylation site on the C_H2 domain important for complement activation and FcγR binding [50]. The mAb lacking this site was defective in complement-mediated cytotoxicity and showed a reduced, although still significant, ability to reduce viral titer in lungs of infected mice.

Conclusion

Respiratory syncytial virus represents a major public health issue, particularly affecting children under the age of 6 months [1]. With point-of-care diagnostics available for detection of this highly contagious virus [51], there is a clear opportunity for early therapeutic intervention to substantially reduce both acute and long-term morbidity associated with RSV infection. A particularly promising target for such therapy is the RSV G-protein, which is involved in active misdirection of the host innate immune response. In rodent models, a mAb against the RSV G-protein has shown efficacy both in reducing viral load and in ameliorating virus-induced immune dysfunction.

Future perspective

Since the time course of RSV infection in rodents is different to that in humans, full assessment of the utility of anti-G mAb therapy awaits clinical testing. Toward that end,

high-affinity native human mAbs to the key epitope on the RSV G-protein have recently been described [14]. Clinical data may be available for this novel class of therapeutic in the next 2–3 years. If effective, a shift in vaccine strategy towards neutralization of the RSV G-protein is likely. Vaccines are most promising for the elderly population, since the immune response in infants appears to be too slow and too weak to be clinically useful. A key issue in RSV biology still to be resolved is the mechanism underlying the poor immunological memory to RSV. Insights into this phenomenon will be helpful for guiding vaccine efforts, particularly in the context of developing an attenuated live virus versus a recombinant subunit approach.

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Bibliography

Papers of special note have been highlighted as:

▪ of interest

▪▪ of considerable interest

- 1▪▪. Hall CB, Weinberg GA, Iwane MK, et al. The burden of respiratory syncytial virus infection in young children. *N Engl J Med* 2009;360(6):588–598. Major epidemiology study that has defined the unmet pediatric clinical need. [PubMed: 19196675]
2. Centers for Disease Control and Prevention (CDC). Respiratory syncytial virus activity – United States, July 2008–December 2009. *MMWR Morb Mortal Wkly Rep* 2009;59(8):230–233.
3. Oshansky CM, Zhang W, Moore E, Tripp RA. The host response and molecular pathogenesis associated with respiratory syncytial virus infection. *Future Microbiol* 2009;4:279–297. [PubMed: 19327115]
4. Mejias A, Chavez-Bueno S, Gomez AM, et al. Respiratory syncytial virus persistence: evidence in the mouse model. *Pediatr Infect Dis J* 2008;27(10 Suppl):S60–S62. [PubMed: 18820580]
5. Mohapatra SS, Boyapalle S. Epidemiologic, experimental, and clinical links between respiratory syncytial virus infection and asthma. *Clin Microbiol Rev* 2008;21(3):495–504. [PubMed: 18625684]
6. Thomsen SF, van der Sluis S, Stensballe LG, et al. Exploring the association between severe respiratory syncytial virus infection and asthma: a registry-based twin study. *Am J Respir Crit Care Med* 2009;179(12):1091–1097. [PubMed: 19286626]
7. Simoes EA, Groothuis JR, Carbonell-Estrany X, et al. Palivizumab prophylaxis, respiratory syncytial virus, and subsequent recurrent wheezing. *J Pediatr* 2007;151(1):34–42. 42, E31. [PubMed: 17586188]
8. Empey KM, Peebles RS Jr, Kolls JK. Pharmacologic advances in the treatment and prevention of respiratory syncytial virus. *Clin Infect Dis* 2010;50(9):1258–1267. [PubMed: 20235830]
9. Somers CC, Ahmad N, Mejias A, et al. Effect of dexamethasone on respiratory syncytial virus-induced lung inflammation in children: results of a randomized, placebo controlled clinical trial. *Pediatr Allergy Immunol* 2009;20(5):477–485. [PubMed: 19397752]
10. Power UF. Respiratory syncytial virus (RSV) vaccines – two steps back for one leap forward. *J Clin Virol* 2008;41(1):38–44. [PubMed: 18340669]
11. Tripp RA, Jones LP, Haynes LM, Zheng H, Murphy PM, Anderson LJ. CX3C chemokine mimicry by respiratory syncytial virus G glycoprotein. *Nat Immunol* 2001;2(8):732–738. [PubMed: 11477410]

12. Zhang W, Choi Y, Haynes L, et al. Vaccination to induce antibodies blocking the CX3C–CX3CR1 interaction of respiratory syncytial virus G protein reduces pulmonary inflammation and virus replication in mice. *J Virol* 2010;84(2):1148–1157. [PubMed: 19864390]
13. Haynes LM, Caidi H, Radu GU, et al. Therapeutic monoclonal antibody treatment targeting respiratory syncytial virus (RSV) G protein mediates viral clearance and reduces the pathogenesis of RSV infection in BALB/c mice. *J Infect Dis* 2009;200(3):439–447. [PubMed: 19545210]
14. Collarini EJ, Lee FE, Foord O, et al. Potent high-affinity antibodies for treatment and prophylaxis of respiratory syncytial virus derived from B cells of infected patients. *J Immunol* 2009;183(10):6338–6345. A 1 pM affinity antibody to respiratory syncytial virus (RSV) G-protein was isolated from human B cells of recovering patients, providing a promising candidate for a new therapeutic agent. [PubMed: 19841167]
15. Cowton VM, McGivern DR, Fearn R. Unravelling the complexities of respiratory syncytial virus RNA synthesis. *J Gen Virol* 2006;87(Pt 7):1805–1821. [PubMed: 16760383]
16. Collins PL, Murphy BR. New generation live vaccines against human respiratory syncytial virus designed by reverse genetics. *Proc Am Thorac Soc* 2005;2(2):166–173. [PubMed: 16113487]
17. Rawling J, Melero JA. The use of monoclonal antibodies and lectins to identify changes in viral glycoproteins that are influenced by glycosylation: the case of human respiratory syncytial virus attachment (G) glycoprotein. *Methods Mol Biol* 2007;379:109–125. [PubMed: 17502674]
18. Pichavant, M.; Goya, S.; Hamelmann, E.; Gelfand, EW.; Umetsu, DT. *Curr Protoc Immunol*. Vol. Chapter 15. 2007. Animal models of airway sensitization; p. 18
19. Wegmann M. Th2 cells as targets for therapeutic intervention in allergic bronchial asthma. *Expert Rev Mol Diagn* 2009;9(1):85–100. [PubMed: 19099351]
20. Legg JP, Hussain IR, Warner JA, Johnston SL, Warner JO. Type 1 and type 2 cytokine imbalance in acute respiratory syncytial virus bronchiolitis. *Am J Respir Crit Care Med* 2003;168(6):633–639. [PubMed: 12773328]
21. Reed JL, Brewah YA, Delaney T, et al. Macrophage impairment underlies airway occlusion in primary respiratory syncytial virus bronchiolitis. *J Infect Dis* 2008;198(12):1783–1793. Comparison of lung histopathology between fatal cases of RSV in infants on the one hand and mice with impaired macrophages and on the other hand highlighted the importance of the innate immune response to the clinical pathology associated with RSV. [PubMed: 18980502]
22. Becker Y. Respiratory syncytial virus (RSV) evades the human adaptive immune system by skewing the Th1/Th2 cytokine balance toward increased levels of Th2 cytokines and IgE, markers of allergy – a review. *Virus Genes* 2006;33(2):235–252. [PubMed: 16972040]
23. Johnson TR, Johnson JE, Roberts SR, Wertz GW, Parker RA, Graham BS. Priming with secreted glycoprotein G of respiratory syncytial virus (RSV) augments interleukin-5 production and tissue eosinophilia after RSV challenge. *J Virol* 1998;72(4):2871–2880. [PubMed: 9525607]
24. Maher CF, Hussell T, Blair E, Ring CJ, Openshaw PJ. Recombinant respiratory syncytial virus lacking secreted glycoprotein G is attenuated, non-pathogenic but induces protective immunity. *Microbes Infect* 2004;6(12):1049–1055. [PubMed: 15380773]
25. Bukreyev A, Yang L, Fricke J, et al. The secreted form of respiratory syncytial virus G glycoprotein helps the virus evade antibody-mediated restriction of replication by acting as an antigen decoy and through effects on Fc receptor-bearing leukocytes. *J Virol* 2008;82(24):12191–12204. High level of soluble G-protein was shown to reduce the efficacy of antibodies to both the F- and G-proteins, indicating a deeper role in modulation of the host immune response than can be accounted for simply by acting as a sponge for anti-G antibodies. [PubMed: 18842713]
26. Castilow EM, Olson MR, Varga SM. Understanding respiratory syncytial virus (RSV) vaccine-enhanced disease. *Immunol Res* 2007;39(1–3):225–239. [PubMed: 17917067]
27. Oshansky CM, Krunkosky TM, Barber J, Jones LP, Tripp RA. Respiratory syncytial virus proteins modulate suppressors of cytokine signaling 1 and 3 and the type I interferon response to infection by a toll-like receptor pathway. *Viral Immunol* 2009;22(3):147–161. Mechanisms by which the RSV G-protein suppresses an effective host immune response include distortion of the innate immune response. [PubMed: 19435411]

28. Shingai M, Azuma M, Ebihara T, et al. Soluble G protein of respiratory syncytial virus inhibits Toll-like receptor 3/4-mediated IFN-beta induction. *Int Immunol* 2008;20(9):1169–1180. [PubMed: 18611945]
29. Gonzalez PA, Prado CE, Leiva ED, et al. Respiratory syncytial virus impairs T cell activation by preventing synapse assembly with dendritic cells. *Proc Natl Acad Sci USA* 2008;105(39):14999–15004. RSV subversion of the host immune response acts at several levels, including interference with T-cell activation by infected dendritic cells. [PubMed: 18818306]
30. Monick MM, Yarovinsky TO, Powers LS, et al. Respiratory syncytial virus up-regulates TLR4 and sensitizes airway epithelial cells to endotoxin. *J Biol Chem* 2003;278(52):53035–53044. [PubMed: 14565959]
31. Polack FP, Irusta PM, Hoffman SJ, et al. The cysteine-rich region of respiratory syncytial virus attachment protein inhibits innate immunity elicited by the virus and endotoxin. *Proc Natl Acad Sci USA* 2005;102(25):8996–9001. [PubMed: 15956195]
32. Dalpke A, Heeg K, Bartz H, Baetz A. Regulation of innate immunity by suppressor of cytokine signaling (SOCS) proteins. *Immunobiology* 2008;213(3–4):225–235. [PubMed: 18406369]
33. Umehara H, Bloom E, Okazaki T, Domae N, Imai T. Fractalkine and vascular injury. *Trends Immunol* 2001;22(11):602–607. [PubMed: 11698220]
34. Tripp RA, Oshansky C, Alvarez R. Cytokines and respiratory syncytial virus infection. *Proc Am Thorac Soc* 2005;2(2):147–149. [PubMed: 16113483]
35. Amanatidou V, Sourvinos G, Apostolakis S, Tsilimigaki A, Spandidos DA. T280M variation of the CX3C receptor gene is associated with increased risk for severe respiratory syncytial virus bronchiolitis. *Pediatr Infect Dis J* 2006;25(5):410–414. [PubMed: 16645504]
36. Tal G, Mandelberg A, Dalal I, et al. Association between common Toll-like receptor 4 mutations and severe respiratory syncytial virus disease. *J Infect Dis* 2004;189(11):2057–2063. [PubMed: 15143473]
37. Mailaparambil B, Krueger M, Heinze J, Forster J, Heinzmann A. Polymorphisms of Toll like receptors in the genetics of severe RSV associated diseases. *Dis Markers* 2008;25(1):59–65. [PubMed: 18776592]
38. Miyairi I, DeVincenzo JP. Human genetic factors and respiratory syncytial virus disease severity. *Clin Microbiol Rev* 2008;21(4):686–703. Two genetic polymorphisms that affect known targets of the RSV G-protein are among the epidemiological risk factors for severe RSV disease. [PubMed: 18854487]
39. Plotnicky-Gilquin H, Cyblat-Chanal D, Goetsch L, et al. Passive transfer of serum antibodies induced by BBG2Na, a subunit vaccine, in the elderly protects SCID mouse lungs against respiratory syncytial virus challenge. *Virology* 2002;303(1):130–137. [PubMed: 12482664]
40. Harcourt J, Alvarez R, Jones LP, Henderson C, Anderson LJ, Tripp RA. Respiratory syncytial virus G protein and G protein CX3C motif adversely affect CX3CR1⁺ T cell responses. *J Immunol* 2006;176(3):1600–1608. [PubMed: 16424189]
41. Ramilo O. Evolution of prophylaxis: MoAb, siRNA, vaccine, and small molecules. *Paediatr Respir Rev* 2009;10(Suppl 1):23–25. [PubMed: 19651397]
42. Carbonell-Estrany X, Simoes EA, Dagan R, et al. Motavizumab for prophylaxis of respiratory syncytial virus in high-risk children: a noninferiority trial. *Pediatrics* 2010;125(1):E35–E51. [PubMed: 20008423]
43. Wu H, Pfarr DS, Johnson S, et al. Development of motavizumab, an ultra-potent antibody for the prevention of respiratory syncytial virus infection in the upper and lower respiratory tract. *J Mol Biol* 2007;368(3):652–665. [PubMed: 17362988]
44. Gill MA, Welliver RC. Motavizumab for the prevention of respiratory syncytial virus infection in infants. *Expert Opin Biol Ther* 2009;9(10):1335–1345. [PubMed: 19764889]
45. Georgescu G, Chemaly RF. Palivizumab: where to from here? *Expert Opin Biol Ther* 2009;9(1):139–147. [PubMed: 19063700]
46. Lagos R, DeVincenzo JP, Munoz A, et al. Safety and antiviral activity of motavizumab, a respiratory syncytial virus (RSV)-specific humanized monoclonal antibody, when administered to RSV-infected children. *Pediatr Infect Dis J* 2009;28(9):835–837. [PubMed: 19636278]

47. Taylor G, Stott EJ, Bew M, et al. Monoclonal antibodies protect against respiratory syncytial virus infection in mice. *Immunology* 1984;52(1):137–142. [PubMed: 6201436]
48. Walsh EE, Hall CB, Schlesinger JJ, Brandriss MW, Hildreth S, Paradiso P. Comparison of antigenic sites of subtype-specific respiratory syncytial virus attachment proteins. *J Gen Virol* 1989;70(Pt 11):2953–2961. [PubMed: 2479715]
49. Miao C, Radu GU, Caidi H, Tripp RA, Anderson LJ, Haynes LM. Treatment with respiratory syncytial virus G glycoprotein monoclonal antibody or F(ab')₂ components mediates reduced pulmonary inflammation in mice. *J Gen Virol* 2009;90(Pt 5):1119–1123. Clear distinction between direct antiviral activity of a monoclonal antibody against RSV G-protein and the effect on airway inflammation was established by molecular dissection of the antibody. [PubMed: 19264600]
50. Mekseepralard C, Toms GL, Routledge EG. Protection of mice against human respiratory syncytial virus by wild-type and aglycosyl mouse-human chimaeric IgG antibodies to subgroup-conserved epitopes on the G glycoprotein. *J Gen Virol* 2006;87(Pt 5):1267–1273. [PubMed: 16603529]
51. Borek AP, Clemens SH, Gaskins VK, Aird DZ, Valsamakis A. Respiratory syncytial virus detection by Remel Xpect, Binax Now RSV, direct immunofluorescent staining, and tissue culture. *J Clin Microbiol* 2006;44(3):1105–1107. [PubMed: 16517905]

Figure 1. High efficacy of anti-G monoclonal antibodies for postinfection treatment
RSV (strain Long) was administered intranasally to Balb/c mice at day 0. The indicated humanized (anti-F: palivizumab) or fully human (anti-G: mAb 3D3; normal IgG: nonimmune isotype control) antibodies were administered intraperitoneally at 5 mg/kg at day 3 postinfection, a dose sufficient to abolish viral load when given prophylactically. Antiviral efficacy was measured at day 5 and day 7 postinfection by qPCR. The anti-G mAb was significantly more potent at reducing viral load in the lungs ($p = 0.076$). Not shown: similar results were obtained when treating at day 5 and measuring at day 7. mAb: Monoclonal antibody; qPCR: Quantitative PCR; RSV: Respiratory syncytial virus. Data taken with permission from [14].

Figure 2. Fc-independent anti-inflammatory activity of anti-G monoclonal antibodies
RSV (strain A2) was administered intranasally to Balb/c mice at day 0. The indicated murine anti-RSV G monoclonal antibody, anti-G F(ab')₂ fragment or nonimmune antibody isotype control were administered intraperitoneally at 150–300 µg/mouse at day 3 postinfection. Two parameters of efficacy (lung viral titers and BAL cell infiltration) were measured at day 5 postinfection. The anti-G F(ab')₂ fragments (missing the Fc portion) had negligible antiviral activity as compared with the full IgG (**A**), but was comparably effective at reducing a key measure of lung inflammation (**B**).
BAL: Bronchoalveolar lavage; pfu: Plaque forming unit; RSV: Respiratory syncytial virus. Data taken with permission from [49].