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Structural basis of respiratory syncytial virus neutralization by motavizumab

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Abstract

Motavizumab is ~tenfold more potent than its predecessor, palivizumab (Synagis), the FDA-approved monoclonal antibody used to prevent respiratory syncytial virus (RSV) infection. The structure of motavizumab in complex with a 24-residue peptide corresponding to its epitope on the RSV fusion (F) glycoprotein reveals the structural basis for this greater potency. Modeling suggests that motavizumab recognizes a different quaternary configuration of the F glycoprotein than that observed in a homologous structure.

Respiratory syncytial virus (RSV) is a highly contagious member of the Paramyxoviridae family of negative-sense RNA viruses. It is estimated that RSV causes 64 million illnesses and 160,000 deaths each year worldwide¹. RSV infects people repeatedly throughout life and causes morbidity in healthy children and adults². Because there is currently no licensed RSV vaccine, passive immunization is used to prevent RSV infection, especially in infants with prematurity, bronchopulmonary dysplasia or congenital heart disease. Originally, RSV-neutralizing polyclonal antibodies from pooled human sera (RespiGam) were used³. This was followed by the development of palivizumab⁴ (Synagis), the only anti-infective monoclonal antibody currently approved by the US Food and Drug Administration (FDA). Palivizumab was humanized from mouse antibody 1129, which binds a 24-residue, linear, conformational epitope on the RSV fusion (F) glycoprotein^{5–7}. When administered at a dose of 15 mg kg⁻¹ each month during the RSV season, palivizumab reduces RSV-related hospitalizations by 55%⁸.

To enhance the potency of palivizumab, each residue in its six complementarity-determining regions (CDRs) was individually substituted with the other 19 residues (a total number of 1,121 unique single variants were assayed), and combinations of beneficial substitutions

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Accession codes. Protein Data Bank: The atomic coordinates and structure factors for the motavizumab–peptide complex have been deposited under accession code 3IXT.

Note: Supplementary information is available on the Nature Structural & Molecular Biology website.

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were evaluated^{9,10}. This led to the development of a second-generation antibody, motavizumab, which is currently in phase III clinical trials and is ~10 times more potent than palivizumab⁹. Only 13 residues differ between motavizumab and palivizumab. Of these, seven individually increase the affinity of the antibody to the F glycoprotein, resulting in a K_d of 0.035 nM (versus 1.4 nM for palivizumab)^{4,9,10}. Here we characterize the structural basis of motavizumab affinity, model mutations with enhanced affinity, and investigate structural implications for motavizumab binding in the trimeric F glycoprotein context.

We used recombinant motavizumab IgG molecules that were shown to neutralize RSV potently (Fig. 1a) to create antigen-binding fragments (Fabs) for crystallographic analysis (Supplementary Methods). Crystals of the Fab were obtained in complex with a 24-residue peptide that corresponds to residues 254–277 of the RSV F glycoprotein A2 strain (NSELLSLINDMPITNDQKKLMSNN) and represents the known epitope for palivizumab and motavizumab⁶. The crystals diffracted X-rays to 2.75 Å, and we obtained a molecular replacement solution containing two molecules per asymmetric unit of the previously determined unliganded palivizumab structure¹¹. Initial maps showed two regions of well-defined helical density near the CDRs of each Fab. We modeled these regions as the peptide, and the structure refined to an $R_{\text{crys}}/R_{\text{free}} = 21.3\%/27.4\%$ (Supplementary Table 1). The peptide forms a helix-loop-helix (Fig. 1b), in agreement with secondary structure predictions for the RSV F glycoprotein¹². The main chain electron density for the peptide was strong for all residues, and the side chain density was strong for residues 262–276 but weak or nonexistent for residues N- and C-terminal to this region (Supplementary Fig. 1). The variable domains of the peptide-bound motavizumab structure and the unbound palivizumab structure were similar (r.m.s. deviation 1.8 Å for variable domain C α), with the largest differences occurring in the three heavy chain CDRs.

To understand the structural basis for the high-affinity interaction between motavizumab and RSV F glycoprotein, we analyzed the structure of the peptide–Fab complex. The interface between the peptide and Fab buries a total of 1,304 Å² of surface area (680 Å² on the peptide and 624 Å² on the Fab, as calculated by PISA¹³) and has a shape complementarity (S_c) value of 0.76, which is substantially higher than the typical range of 0.64–0.68 for antibody–antigen complexes¹⁴. The electrostatic potentials on the surface of the peptide and Fab are also complementary, with several acidic patches on the Fab interacting with positively charged regions on the peptide (Supplementary Fig. 2). Approximately 73% of the surface area buried on the Fab is located on the heavy chain, which possesses a large hydrophobic region consisting of residues from the second and third CDRs (Fig. 1c). This region contacts peptide residues located along the length of both helices. The four peptide residues between the two helices do not contribute to motavizumab binding, having only 8 Å² buried at the interface. Interactions between the peptide and heavy chain include hydrogen bonds formed between the peptide side chain of Asn262 and the Fab side chains of Asp54 and Lys56 as well as a hydrogen bond between the peptide side chain of Ser275 and the carbonyl oxygen of Fab residue Ile97. There are also several interactions between the peptide and light chain. These include a hydrogen bond between the side chain of peptide

residue Asn268 and the carbonyl oxygen of Gly90 as well as a salt bridge between the peptide side chain of Lys272 and the side chain of Asp49 in the second CDR (Fig. 1c).

The interactions between the peptide and motavizumab Fab are consistent with RSV F glycoprotein mutations known to disrupt antibody binding to this epitope. It has been shown that mutations N262Y, N268I and K272E decrease the binding of several antibodies that recognize this region of the F glycoprotein⁶. The mutations K272M and K272Q have also been found in RSV F glycoprotein escape mutants that are resistant to palivizumab¹⁵. The side chains of these three peptide residues all form hydrogen bonds or salt bridges with residues in motavizumab (Fig. 1c).

To investigate the structural basis for motavizumab's enhanced potency over palivizumab, the positions of the seven altered residues that increase the affinity to the F glycoprotein were analyzed in the peptide-bound crystal structure (Fig. 1d). Three of the seven altered residues (S32A, T98F and W100F in the heavy chain) directly contact the peptide and are located in the hydrophobic patch described earlier. Both S32A and T98F substitutions increase the hydrophobicity of this patch, favoring interactions with the peptide. As for the W100F mutation, the smaller phenylalanine side chain is able to pack tightly against peptide residues Asn268 and Lys272. The larger tryptophan side chain found in palivizumab would likely alter the conformation of these residues, which make hydrogen bond and salt bridge interactions with the Fab, respectively. When the three palivizumab residues were modeled into the complex, the S_c value decreased from 0.76 to 0.70, reflecting the poorer fit between the peptide and Fab.

The other four substituted residues that increase the potency of motavizumab do not contact the peptide directly. Two of the mutations (D58H and S95D in the heavy chain) are located near the interface with the peptide, and their side chains interact with other residues in the CDRs. Thus, they likely exert their effects indirectly by altering the position of other residues that do contact the peptide. The side chains of the two remaining substitutions, S65D in the heavy chain and S29R in the light chain, have weak electron density and do not contact any residues in the peptide or Fab. However, both substitutions increase the on-rate of motavizumab for the F glycoprotein, and the S29R mutation alone results in a 4.4-fold increase in RSV neutralization *in vitro*⁹. Collectively, these data suggest that the S65D and S29R side chains either bind to residues in the F glycoprotein located outside the primary epitope or increase favorable long-range electrostatic interactions. Relevant to this, we note that motavizumab binds to the peptide ~6,000-fold more weakly than the full-length F protein (230 nM versus 0.035 nM)^{9,16}, though some fraction of the decrease in peptide affinity is likely due to the peptide not adopting the helix-loop-helix conformation in solution⁷.

An earlier version of motavizumab contained residues Phe52, Phe53 and Asp55 in the light chain CDR2, which increased *in vitro* RSV neutralization ~twofold⁹. However, these residues also increased nonspecific tissue binding and decreased the *in vivo* potency⁹, perhaps due to the two solvent-exposed phenylalanine residues (Fig. 1d). Thus, they were ultimately returned to the residues found in palivizumab (Ser52, Lys53 and Ala55).

To visualize the binding of motavizumab to the full-length F glycoprotein, a model was generated based on the pre-fusion parainfluenza virus 5 (PIV5) structure¹⁷ (12.4% sequence identity to RSV F12). Sequence alignment (Supplementary Fig. 3a) identified a similar helix-loop-helix, and structural analysis provided a precise alignment (Supplementary Fig. 3b), shifting the PIV5 sequence by three residues to provide a superposition of the motavizumab epitope and PIV5 of 2.1-Å r.m.s. deviation for all 24 peptide C α atoms (Fig. 2a). A model was generated by orienting the Fab via superposition of the bound peptide onto the corresponding epitope in the PIV5 F glycoprotein structure. The resulting model shows no clashes between the Fab and the F glycoprotein monomer to which it is bound (Fig. 2b).

In the pre-fusion trimeric context, however, both the heavy and light chains of the Fab clash with an adjacent RSV F monomer that packs against the same face of the helix-loop-helix that motavizumab binds (Fig. 2c,d). The location of the epitope at a subunit interface may explain why this neutralizing epitope is so highly conserved in RSV strains. Because motavizumab neutralizes RSV by preventing the fusion of the viral and cellular membranes⁴, motavizumab must bind to the F glycoprotein before or during the transition to the post-fusion state. The extensive clashes in the trimer model, however, suggest that motavizumab would be unable to bind the pre-fusion trimeric F glycoprotein as it exists in the PIV5 structure. To address this issue experimentally, we expressed and purified a soluble RSV F glycoprotein in a form similar to the PIV5 F glycoprotein used in the modeling. Specifically, the furin cleavage sites were mutated and a fibritin trimerization motif¹⁸ was appended to the truncated C terminus to keep the protein in a trimeric, pre-fusion conformation. This stabilized RSV F glycoprotein, referred to as RSV F₀ Fd, eluted from a gel filtration column with a retention volume consistent with that of a glycosylated trimer (Fig. 2e). To determine whether motavizumab or palivizumab is able to bind the pre-fusion trimeric RSV F glycoprotein, we added palivizumab Fab in excess to a solution of RSV F₀ Fd and then passed the mixture over a gel filtration column. The elution profile contained two peaks, corresponding to excess Fab and a complex of the Fab and F glycoprotein (Fig. 2f). The elution volume of the complex peak was consistent with a trimeric F glycoprotein bound by three Fabs, in agreement with the ratio (1:2.97) of F glycoprotein and Fab bands observed on a Coomassie blue-stained SDS-PAGE gel containing fractions from the complex peak (Fig. 2f).

Collectively, these data suggest that motavizumab binds to or induces a conformation of the trimeric F glycoprotein that is different from the one observed in the PIV5 F pre-fusion structure. One possibility is that the structure of the RSV F glycoprotein differs substantially from that of PIV5, although the predicted RSV F glycoprotein secondary structure appears similar to that observed in the PIV5 F pre-fusion crystal structure (Supplementary Fig. 3). Another possibility is that motavizumab traps an intermediate between pre- and post-fusion forms. It has been suggested that during this transition, which is one of the largest structural rearrangements known, the F glycoprotein monomers transiently dissociate before forming the trimeric post-fusion conformation¹⁷. We note, in this regard, that glutaraldehyde cross-linking of the soluble F glycoprotein trimer does not inhibit motavizumab binding (Supplementary Fig. 4). Alternatively, the RSV F glycoprotein in its pre-fusion conformation may have sufficient flexibility to bind three motavizumab Fabs. Modeling studies indicate that a ~30° rotation of domain III parallel to the threefold axis would allow

clash-free binding of three Fabs. A similar degree of rotation has been observed in cryo-EM tomograms after the binding of neutralizing antibodies to dengue virus¹⁹ and HIV-1 (ref. 20). Such flexibility may be a more common feature of viral fusion proteins than previously thought.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Structural basis of motavizumab binding to its F glycoprotein epitope. **(a)** RSV neutralization curves for palivizumab and motavizumab IgG as determined by a flow cytometric assay. **(b)** Surface representation of the Fab and ribbon representation of the peptide, viewed looking down at the CDRs and a 90° rotation about the horizontal axis from **c** and **d**. **(c)** Interactions between the peptide and six Fab CDRs. Side chains are shown for those residues making intermolecular interactions. Dashed lines represent hydrogen bonds. Residues in the hydrophobic patch on the heavy chain are shown with transparent surfaces. These include Trp52 and Trp53 on the right and Ile97, Phe98 and Phe100 on the left. **(d)** Motavizumab mutations that alter affinity to the F glycoprotein. Side chains are shown for those Fab residues that increase affinity by directly contacting the peptide (red), by altering the position of residues that directly contact the peptide (cyan) or by binding to the F glycoprotein outside the primary epitope or enhancing long-range electrostatic interactions (magenta). Fab residues in yellow decrease the affinity for the F glycoprotein but increase *in vivo* potency compared to an earlier version of motavizumab.

Figure 2.

Motavizumab binding to RSV F glycoprotein. **(a)** Superposition of the motavizumab-bound peptide (gray) and residues 229–252 of the PIV5 F glycoprotein structure (red). **(b)** Ribbon representation of the model of motavizumab Fab (green and blue) bound to the PIV5 F glycoprotein monomer (tan) via the superposition shown in **a**. **(c)** Same as **b**, except the entire PIV5 F glycoprotein trimer is shown (tan, green, pink). **(d)** Magnification of the boxed area shown in **c**. **(e)** Gel filtration elution profile and corresponding Coomassie blue–stained SDS-PAGE gel of the RSV F₀ Fd glycoprotein. **(f)** Gel filtration elution profile and corresponding Coomassie blue–stained SDS-PAGE gel of a mixture of RSV F₀ Fd glycoprotein and excess palivizumab Fab. Densitometric analysis of the gel yields a ratio of 2.97 Fabs per trimer.