Identification and Human Serum Reactogenicity of Neutralizing Epitopes within the Central Unglycosylated Region of the Respiratory Syncytial Virus Attachment Protein

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Running title: Neutralizing Epitopes of RSV G Protein

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Abstract:

We identified two overlapping neutralizing epitopes within residues 151-172 of the central unglycosylated region of the RSV attachment protein. In ~40% of hospitalized and outpatient adults infected with RSV subtype A, these contiguous residues are the target of ≥ 4-fold increase in IgG response between acute and convalescent sera.
A hallmark of the respiratory syncytial virus (RSV) attachment (G) protein is the central unglycosylated region that is typically comprised of amino acids (aa) 151-190 (9). Residues 164 to 176 are invariantly conserved among G sequences from all RSV isolates and there is a “loop” structure comprised of two cystine disulfide bonds (Figure 1). Genetic selection for RSV that can replicate in the presence of subtype-independent, partially neutralizing anti-G monoclonal antibodies (mAbs) such as L9 yields strains bearing aa changes mostly localized within the central unglycosylated region of G (8, 14).

To better define the cognate epitopes for L9 as well as for the K6 mAb with similar neutralizing activities, we constructed a series of plasmids, each encoding a glutathione S-transferase (GST)-RSV G fusion protein bearing a portion of the central unglycosylated region of G ((15); Supplemental File). The fusion proteins were expressed in bacteria (Supplemental File), purified to >95% homogeneity (data not shown), resolved on sodium dodecyl sulfate (SDS)-polyacrylamide gels under reducing and denaturing conditions, and tested for recognition by L9 and K6 mAbs in immunoblots.

L9 and K6 did not recognize GST alone or to GST-G173-190, but bound to purified, full-length G protein, GST-G151-190, and three derivatives of GST-G151-172, each bearing corresponding residues from A2, B1, or RGH strains (Figure 2). Neither mAb recognized GST-G151-161 but GST-G155-172, -G157-172, and -G162-172 was detected by K6 but not by L9 (Figure 2 and data not shown). Consistent with these immunoblot...
results, all three GST-G151-172 were recognized by both mAbs and GST-G162-172 was bound by K6 but not L9 in enzyme-linked immunosorbent assays (ELISAs) under non-reducing/denaturing conditions (data not shown). These results suggest that: 1) the RSV G residues 151-172 are required for recognition by L9; 2) consistent with the subtype-independent neutralizing activity of both mAbs, K6 and L9 both recognized subtype A- and B-derived residues 151-172; and 3) the K6 epitope involves aa 162-172. Based on the proximal location with respect to the G loop, we have termed residues 151-172 as the “proximal central core” (PCC) region of the RSV G protein.

The RSV G PCC contains most of the conserved aa HFEVFNFVPCSIC (residues 164-176). Our data suggest that the K6 epitope likely exists within these residues while the L9 epitope requires non-conserved residues 151-163 for correct epitope conformation.

Also within the PCC is the motif Y/F XFXXFXF (residues 163-170), in which F163, F165, F168, and F170 are present in G proteins from subtype A strains (e.g. A2, RGH) while Y163 is noted in the G protein from the B1 strain ((5, 16); Figure 1). This aa motif is also found in RSV-related viruses of different host specificities (e.g. ovine and bovine RSV), suggesting an evolutionarily conserved structural and/or immunological function (6, 7). Alanine substitution of F163 but not of F165 abolishes K6 binding to GST-G162-172 in immunoblots, suggesting that the F163 residue is likely involved in K6-epitope interactions (data not shown).

To determine the clinical and immunological relevance of the PCC-embedded epitopes in human RSV infections, we assayed the serum reactogenicity to GST-G151-172 in
ELISAs using paired acute and convalescent sera from RSV subtype A-infected hospitalized and outpatient adults ((3); Supplemental File). Among paired sera from 32 RSV-infected hospitalized adults, 14 (44%) had a ≥ 4-fold increase in the anti-RSV G PCC titers from acute to convalescent sera. Similarly, in serum samples from 19 outpatient adults, eight (42%) had ≥ 4-fold increase in anti-G PCC antibody response. In both populations, the increase in the mean ± SD (reciprocal log₂) anti-G PCC titers after RSV infection was statistically significant (hospitalized: 8.3 ± 1.8 → 10.1 ± 2.8; outpatient: 8.1 ± 1.5 → 9.9 ± 1.6; for both, p < 0.005 by Wilcoxon sign-rank test). These data suggest that: 1) an acute rise in anti-G PCC titers is found in ~40% of RSV subtype A-infected adults; and 2) such titer increases are observed with no obvious correlation to the severity of illness as defined by the initial clinical evaluation in hospitalized vs. outpatient settings.

Our results have implications for structure, function, and immunogenicity of the RSV G PCC region. Due to hydrophobic interactions involving F165, F168, F170, V171, P155, and P156, aa 149-177 of the RSV G likely forms a disc-like structure with two hydrophobic faces (4). Within the G central unglycoylated domain, residues 166-170 (EVFN) may be involved in multimerization of RSV G and/or interactions with a cellular RSV G protein receptor (4). Our results suggest that ≥1 neutralizing epitope is found within and flanking RSV G residues 166-170 and raise the possibility that L9 and other mAbs recognizing the G PCC-embedded epitopes may directly or indirectly affect RSV G structure (e.g. destabilize multimerization) and/or function (i.e. block interactions with the host target cell). Previously we reported the isolation of an L9-resistant virus.
that bore mutations outside of the G PCC region; perhaps these mutations represent second site/compensatory changes that counteract the action(s) of L9 mAb on RSV G structure/function (14).

Within the RSV G protein, short “protective” B-cell epitopes have been reported, of which two (aa 152-163 and 165-172) are located within the RSV G PCC region (12). It should be noted that L9 and K6 are both neutralizing and subtype-independent, whereas none of the protective epitope-recognizing mAbs were neutralizing and it is unclear whether the protective effect against viral challenge was subtype-independent. These differences in the functional profiles of the various mAbs may be due to the immunogen (purified, native RSV G protein from RSV infected mammalian cells vs. bacterially derived, refolded RSV G fragment) used to generate the respective mAbs.

The human serological characterization of RSV G epitopes, especially those within the G PCC domain, remains incomplete. A very limited number of adult human sera (n=2) were used to study the reactogenicity of RSV G derived “protective” epitopes (12). Other RSV G-based human serological screening studies utilized bacterially synthesized, genetically hypervariable regions flanking the central unglycosylated region or G-derived peptides (overlapping or non-overlapping) to screen adult or pediatric sera (1, 2, 10, 11, 13). In this study, we demonstrate that a ≥ 4-fold increase in serum reactogenicity against the PCC domain of RSV G is noted in a significant proportion of RSV subtype A-infected adults. These data suggest that the G PCC domain is immunologically
significant in human RSV infections and may be a target of prophylactic and/or therapeutic agents against RSV.

Acknowledgements

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References

Figure Legends

Figure 1. The central unglycosylated region of the RSV G protein. Residues 151-190 of the RGH subtype A5 RSV strain and those from the prototypical subtype A (A2) and B (B1) strains. Where relevant, the positions of relevant residues are shown by the numbers above the amino acid alignment. Note that the invariant residues 164-176 are shown in
bold, and the predicted disulfide bonds are shown as solid lines connecting the relevant
cysteine residues. Residues 151-172 ("Proximal Central Core" (PCC); see Text) bearing
the epitopes for L9 and K6 mAbs are dotted underlined.

Figure 2. The cognate epitopes of L9 and K6 mAbs are localized within PCC domain of
the RSV G protein. Panels a-c: representative immunoblots in which purified RSV G
protein (subtype A), GST alone, or GST-RSV G fusion proteins (~0.5µg/protein; G-
derived residues listed above each corresponding lane) were resolved on denaturing
12%/6% SDS-PAGE gels, transferred onto nitrocellulose, and probed with L9 or K6
mAbs at 1:5,000 dilution followed by goat α-mouse HRP conjugated antibodies
(1:20,000 dilution) and chemiluminescence. For each gel, molecular weight (MW)
markers are resolved on the leftmost lane and the corresponding MW masses (in
kilodaltons (kD)) are indicated on the left. Note that in panel (a), two independent
preparations of GST-G173-190, one from DH5α (D) and another from Origami 2 (O)
bacterial strains, were tested for reactogenicity, and that we occasionally observed a
doublet in GST-G151-190 lanes. Note also that in panel (c), aa 151-172 derived from the
RGH, A2, or B1 RSV strains (as shown in lane assignments in parenthesis) were tested
for recognition by L9 and K6 mAbs. (d): The L9 and K6 mAb epitopes are overlapping
within RSV G 151-172. To the right are the various schematic diagrams, each
representing a portion of the RSV G unglycosylated region that was expressed as fusion
proteins in bacteria. Where relevant, the relative locations of the four cysteines ("C") are
shown. To the left is a table summarizing the immunoblot data using both mAbs. (+)
indicates reproducible mAb recognition of the RSV G derived residues while (-) indicates the absence of detectable mAb binding.