Comparison Between Palivizumab Competitive Antibody (PCA) Assay Containing Pre-Fusion, Intermediate, and Post-Fusion Conformation of the Fusion (F) Protein of Respiratory Syncytial Virus (RSV)

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BACKGROUND

1. Respiratory Syncytial Virus (RSV) is recognized as the most common cause of lower respiratory tract infections in young children and an important cause in older adults.

2. Currently, there is no licensed RSV vaccine, despite the huge medical need.

3. Palivizumab is the only FDA approved monoclonal antibody (mAb) for the prevention of severe RSV infection in select high-risk infants.

4. Palivizumab targets antigenic site II of the Fusion (F) protein, which is well conserved among the different RSV genotypes and present in the pre-fusion and post-fusion conformations.

5. Measuring palivizumab-like antibody (PLA) activity might serve as a good immune correlate of protection for RSV vaccine trials.

OBJECTIVES

1. To determine the conformations of four different F proteins (commercially available source; cell-based RSV, and sucrose purified RSV (spRSV) coated on an ELISA plate for 4 or 18 h) using pre-fusion specific mAbs D25 (antigenic site I), 5C4 (site I), and AM14 (site V), and post-fusion specific mAb 131-2A (site I), as well as palivizumab and motavizumab (site II) that are shared by both pre-fusion and post-fusion conformations.

2. To determine if different conformations of F protein used in Palivizumab Competitive Antibody (PCA) assay to measure PLA impact the PLA concentrations in hematopoietic stem cell transplants (HSCT) adults infected with RSV/A and RSV/B.

METHODOLOGY

1. Four PCA assays were developed with the different conformations of F protein described above.

2. The lower limit of detection was 1 µg/mL for three PCA assays, and 20 µg/mL for the cell-based RSV PCA assay.

3. These PCA assays were then used to measure PLA concentration in acute and convalescent serum samples collected from 22 RSV/A and 18 RSV/B infected HSCT adults.

4. PLA concentration (µg/mL) were calculated using Four Parameter Logistic (4PL) Regression model and statistically analyzed by the two-tailed t test and Pearson correlation.

RESULTS

1. Palivizumab competitive antibody (PCA) assay containing pre-fusion, intermediate, and post-fusion conformations of the F protein of respiratory syncytial virus (RSV) measured PLA concentrations in hematopoietic stem cell transplant (HSCT) adults infected with RSV/A and RSV/B.

2. Palivizumab-like antibody concentration in acute and convalescent sera from RSV/A and RSV/B infected HSCT adults by PCA assay.

3. Palivizumab-like antibody concentration in acute and convalescent sera from RSV/A and RSV/B infected HSCT adults measured by PCA assay.

SUMMARY

1. Pre-fusion, post-fusion, and intermediate forms of RSV F protein were identified by a panel of mAbs targeting antigenic sites I, II, and I.

2. The convalescent serum samples had a statistically significant increase in PLA concentration post RSV infection.

3. RSV/A and RSV/B infection induced comparable increase in PLA concentration.

4. Prolonged RSV shedding was associated with significantly reduced PLA concentration post RSV infection.

5. A high correlation was observed between the reference assay (post-fusion F) and the other three PCA assays.

6. A high correlation was observed between PLA concentration measured by any of the 4 PCA assays and neutralizing antibody titer to RSV/A and RSV/B.

Conclusion

- Post-fusion, pre-fusion F and intermediate conformations of F protein used in the PCA assay measured comparable rises in PLA concentration post RSV infection.

- Infections caused by RSV/A and RSV/B produced comparable PLA concentration consistent with antigenic site II being conserved among the RSV/A and RSV/B genotypes.

- Thus, the formation of the F protein should not have a measurable impact on the PCA assay.

FUTURE DIRECTION

Develop a D25 competitive antibody assay (DCA) and evaluate which assay (PCA or DCA) is a better correlate of protection against infection with RSV/A and RSV/B.

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Figure 1. Determination of different conformations of RSV fusion protein by a panel of monoclonal antibodies targeting antigenic sites I, II, and I

Figure 2. Correlation of palivizumab-like antibody activity in sera from RSV/A and RSV/B infected HSCT adults by PCA assay

Figure 3. Correlation of palivizumab-like antibody activity to RSV neutralizing antibody titer in sera from RSV/A and RSV/B infected HSCT adults

Table 1. Palivizumab-like antibody concentration in acute and convalescent sera from RSV/A and RSV/B infected HSCT adults by PCA assay

Table 2. Palivizumab-like antibody concentration in acute and convalescent sera from RSV infected HSCT adults by PCA assay

Table 3. Palivizumab-like antibody concentration in acute and convalescent sera from RSV/A and RSV/B infected HSCT adults by PCA assay