Non-Neutralizing HRV2-Specific Monoclonal Antibody 2G2 Attaches to a Region that Undergoes Most Dramatic Changes Upon Release of the Viral RNA

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Abstract- The monoclonal antibody 2G2 has been extensively used for detection and quantification of structural changes of human rhinovirus serotype 2 (HRV2) during infection. It recognizes exclusively subviral particles produced during infection but not native virus. Since 2G2 is not a neutralizing antibody, viral escape mutants can not be obtained in order to determine the epitope. We have determined the structure of complexes between Fab fragments of 2G2 and 80S subviral B particles using cryo-electron microscopy. Thus we have elucidated the basis of the selectivity of 2G2 by determining its footprint on empty HRV2 capsids. The footprint of the antibody corresponds to the capsid region that we predicted to undergo the most dramatic changes upon RNA release.

1. Introduction

Human rhinoviruses (HRVs), the major cause of the common cold, belong to the family of picornaviruses. They are icosahedral with T=1, pseudo T=3 symmetry, about 30 nm in diameter, and composed of 60 copies of each of the proteins VP1 through VP4 that encapsidate a single stranded positive-sense RNA genome. During infection HRV2 binds to its cell surface receptor (LDL-R) [1], [2] and is internalized into an endosome where the virion undergoes coordinated structural changes preceding the RNA release into the cytosol. The low pH of the late endosomal compartment is the exclusive trigger for these modifications.

2. Results

Using cryo-EM and X-ray structural data we produced a model for the HRV2 empty capsid after RNA release [3]. The capsid was seen to have expanded by 4% with a relative movement of all capsid proteins. In particular, the viral protein VP1 around the 5-fold axes make an iris type of movement to open a 10Å diameter channel, which allows the RNA genome to exit.

The monoclonal antibody 2G2, obtained from a mouse injected with purified HRV2, proved extremely useful for the detection of the structural changes occurring upon infection. For example, it was employed to unequivocally demonstrate the low-pH dependency of HRV2 for infection. However, the binding epitope has remained unknown. We have now solved the structure of complexes between empty capsids of HRV2 and Fab fragments of 2G2 by cryo-electron microscopy [4]. EM and X-ray data of the empty capsid and a similar Fab those structure is known have been to combined to locate the footprint of 2G2. This analysis reveals that the binding epitope of this antibody lies in the region that we have predicted changes most upon the transition between the native virion and the empty capsid [3] Figure 1.

3. Conclusion

The footprint of this antibody thus helps to confirm the cryo-EM model of empty HRV2 and explains the high specificity of this antibody for HRV2 capsids modified during infection.

4. References

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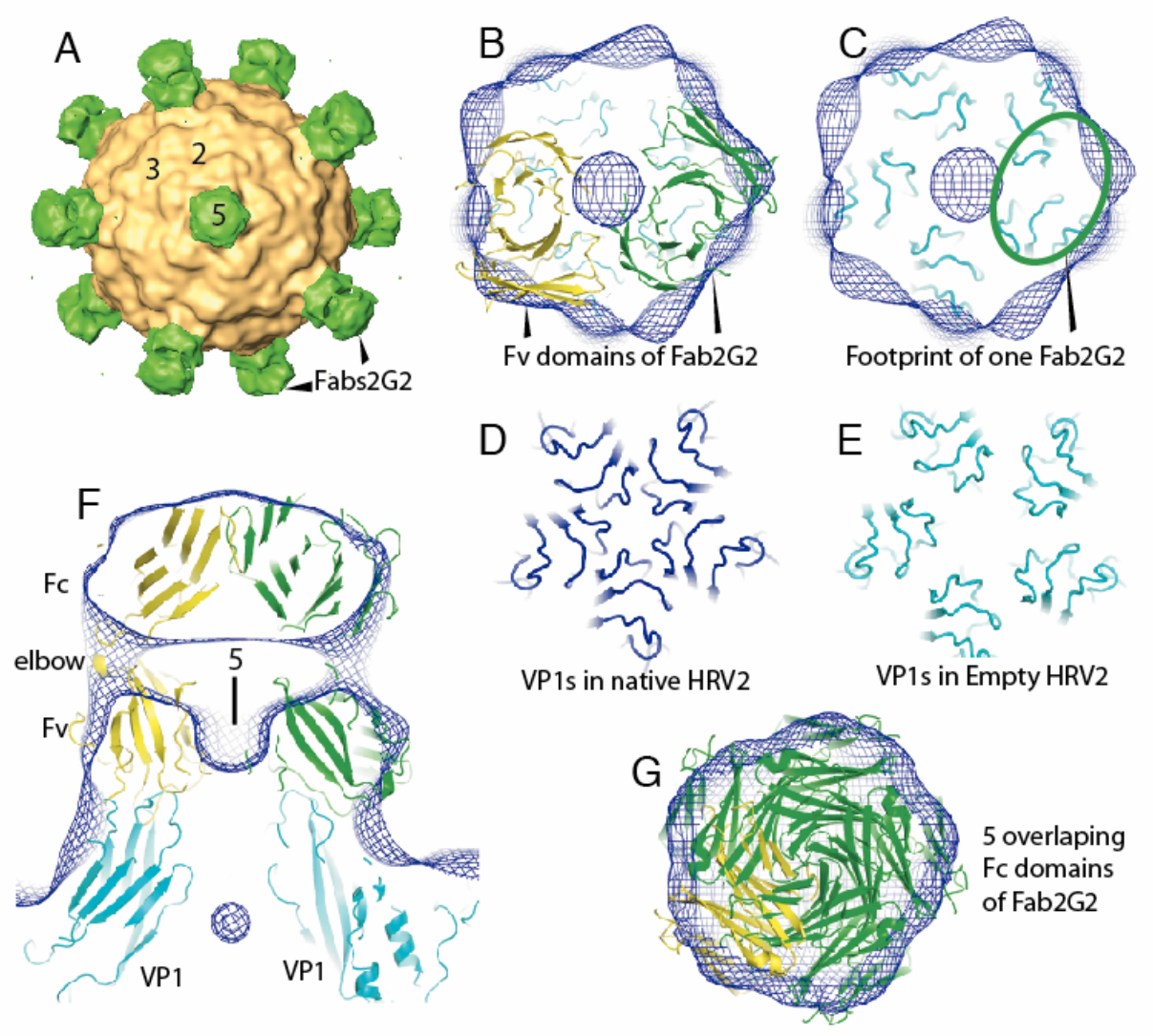

Figure 1. 3D reconstructions of the empty capsid of HRV2 in complex with Fab fragments of 2G2. (A) is a view down a 5-fold axis of the complex with the 2G2 Fabs shaded in green. Representative 2-, 3- and 5-fold icosahedral axes are marked. In the following figures one Fab is shaded yellow and the others are shaded in green. The C_a chains of VP1 in the empty capsid are depicted in cyan. The density map of the complex is represented by a blue mesh. Docking was performed manually in O. A view down a 5-fold axis of a thick section shows two Fab molecules docked in the complex map (B) and the footprint of one Fab which overlaps two VP1 molecules is outlined in green (C). The disposition of the VP1 around a 5-fold axis for the native capsid shaded in dark blue (D) and the empty capsid shaded in cyan (E). (E) shows that each VP1 has moved with respect to its neighbour thus modifying any epitope that covers two VP1. (F) is a thick section parallel to a 5-fold axis showing the fit of the Fab in the density of the complex. (G) shows the fit of the constant domains of the Fab. As for the variable domains steric hindrance is evident and will prevent more than 2 Fabs to bind on each 5-fold axis.
The figures made with PyMOL (http://pymol.sourceforge.net/)