

15th International HIV Drug Resistance Workshop



13-17 June 2006, Sitges, Spain

MOLECULAR DETERMINANTS OF RESISTANCE TO THE gp41-DIRECTED NEUTRALIZING ANTIBODY D5: INSIGHTS FROM A CRYSTAL STRUCTURE OF D5 Fab BOUND TO 5-helix

Antivir Ther. 2006, 11:S19 (abstract no. 14)

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The human monoclonal antibody D5-IgG1 binds to the heptad repeat 1 (HR1) region of the HIV-1 gp41 protein and blocks entry of diverse HIV isolates from multiple subtypes. D5 is thought to block entry by preventing gp41 folding into a “trimer-of-hairpins” conformation in a mechanism analogous to that of T20. We now present data that define the D5 epitope, demonstrate that HR1 is the physiologic target of D5, and begin to address the binding correlates of neutralization.

We performed initial epitope mapping using HR1 mimetics containing alanine substitutions at each external residue (i.e., all but the a and d positions). Binding studies done with these variants showed that gp41 residues L568, W571, and K574 are critical for D5 binding to HR1 *in vitro*; these amino acids overlap a highly conserved hydrophobic pocket. To demonstrate that HR1 binding is critical relevant for the antiviral mechanism-of-action, we engineered alanine substitutions at each of these positions in an HIV proviral clone. Viruses with W571 were not infectious, but both the L568A and K574A viruses were 10-20-fold resistant to D5-IgG. These viruses were fully sensitive to other neutralizing antibodies (2F5, IgG1b12, 2G12) and hypersensitive to inhibitors targeting the prehairpin intermediate (T20, 5-helix). These findings confirm that the D5 epitope defined *in vitro* is the physiologic target of D5.

A high-resolution (2.0 Å) X-ray structure of the D5 Fab bound to 5-helix, another HR1 mimetic, confirmed that D5 binds to an epitope that includes the conserved pocket. Strikingly, a phenylalanine residue from D5 binds in the hydrophobic pocket, mimicking binding of a tryptophan side chain in the endogenous HR2 ligand. D5 amino acids also make direct contacts with HR1 residues L568A, W571 and K574. To address the binding correlates of neutralization, we constructed D5 variants in which key contact residues

were mutated, and then tested the mutant antibodies for antiviral activity and binding kinetics. The data indicate that the off-rate is critical for neutralization: antibodies with a binding $t_{1/2}$ X 5min. retained full neutralizing capacity, whereas an antibody with a $t_{1/2}$ X 2min. was not effective. Future studies will determine whether potency can be improved by improving the on-rate.

Acknowledgments: The HIV Antibody Discovery Team includes Z An¹, G Barbato², E Bianchi², D Bramhill¹, A Carfi², G Ciliberto², J Cook¹, R Cortese², P Di Giovine², D Eckert¹, E Emini¹, AC Finnefrock¹, M Finotto², R Geleziunas¹, R Hampton¹, D Hazuda¹, R Hrin¹, P Ingallinella², S Jarantow¹, J Joyce¹, P Kim¹, S Lane³, S Lennard³, X Liang¹, C Lloyd³, D Lowe³, M Lu¹, P Lu¹, M Luftig², M Mattu², M McElhaugh¹, J Osbourn³, M Patel¹, A Pessi², W Schleif¹, J Shiver¹, W Strohl¹, T Vaughan³, H Zhang¹. ¹Merck Research Laboratories, West Point, PA, USA; ²IRBM P. Angeletti, Pomezia, RM, Italy; ³Cambridge Antibody Technology, Cambridge, UK

2006-06-13
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