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Mechanism of recognition of a membrane-embedded epitope by the anti-HIV broadly-neutralizing 10E8 antibody.

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The mechanism by which the MPER epitope of gp41 of HIV-1 is recognized by the broadly neutralizing antibody 10E8 at membrane interfaces is not known. We determined the crystal structure of 10E8 Fab complex in complex with the transmembrane antigen in detergents. Our research clarifies the molecular mechanisms underlying broad neutralization by 10E8, and guiding future vaccination strategies targeting the MPER antigen of HIV-1 [1].

1 Introduction

Despite of the innumerable advances in virology, pharmacology and clinical fields throughout these years, >30 million people remain infected by HIV according to the WHO. The high mutation rate of HIV-1 and its viral cycle make this virus very difficult to eradicate once it has infiltrated in the human body. The current antiretroviral therapy has turned HIV infection into a chronic disease, but it is unable to completely eradicate the virus.

A promising therapeutic strategy consists in eliciting vaccination responses in which broadly neutralizing antibodies (bNAbs) are generated, although the efficiency of the level of the responses are still unsatisfactory. 10E8 is a potent bNAb very efficient against nearly all known strains of HIV-1 [2]. The advantageous properties of 10E8 could be used to devise novel strategies of anti-HIV-1 vaccination. However, there are fundamental properties of 10E8 that need to be addressed before it can be used efficiently for that purpose. In this study, we determined the influence of the cellular membrane in the mechanism of recognition using lipidic model systems and X-ray crystallography.

2 Experiment

Crystals of the complex between 10E8 Fab (3 mg L^{-1}) and the peptide 10E8ep at a molar ratio of ~1:1.5 (Fab:peptide) in the presence of 2.5 mM detergent dodecylphosphocholine were grown in a solution of 50% (v/v) 2-methyl-2,4-pentanediol, 200 mM phosphate, and 100 mM TRIS (pH 8.5) at 20 °C. A single protein crystal was harvested and stored in liquid nitrogen. Data collection was carried out at beamline AR-NW12 of the Photon Factory in Tsukuba, Japan.

Isothermal titration calorimetry (ITC) was performed with a VP-ITC instrument at 25 °C. Proteins were dialyzed overnight at 4 °C. Fab 10E8 (3 μM) was titrated with peptide (40 μM). For differential scanning calorimetry (DSC) the heat capacity was measured using a VP-DSC scanning microcalorimeter. Samples of 10E8 (10 μM) with and without peptide (15 μM) were heated from 30 to 90 °C at a rate of 1 °C min^{-1}.

3 Results and Discussion

We have determined the structure of 10E8 Fab in complex with the membrane-proximal external region peptide antigen (MPER) and significant portion of the transmembrane domain (TMD) of gp41 (Fig. 1). The dissection of the structural and the energetic factors controlling the recognition of the epitope by the Fab in model membrane systems explains the potent neutralization capabilities of the full-length antibody. We propose that the helical scaffold of the MPER / TMD region of gp41, strengthened by nonpolar interactions with membrane lipids, is critical for the generation of potent anti-MPER broadly neutralizing antibodies [1].

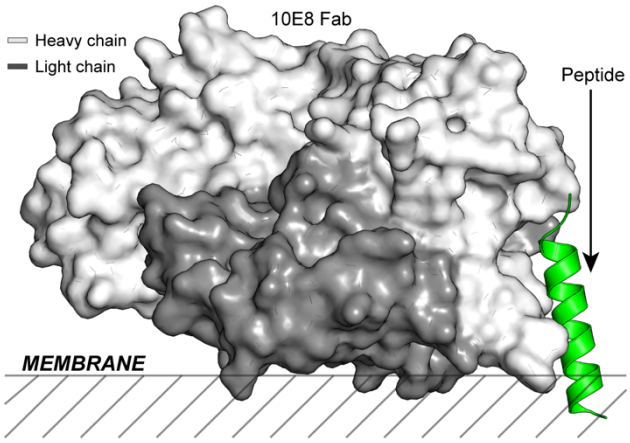

Fig. 1: X-ray structure of the bNAb 10E8 with MPER/TMD peptide in micelles. The position of the membrane with respect to the complex is approximated with the lines at the bottom of the figure.

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References

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