

## STRUCTURE NOTE

# Structure of an Anti-HIV Monoclonal Fab Antibody Fragment Specific to a gp120 C-4 Region Peptide

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**Introduction.** The structures of antibodies raised against peptides representing HIV-1 epitopes are of intrinsic interest in the battle against AIDS. Antibodies that neutralize the virus, effectively stopping its reproduction, are of particular interest because the structure of a neutralizing antibody should contain information concerning the way neutralization can be accomplished. To that end, structures have been published describing neutralizing antibodies raised against representatives of the third hypervariable region (V3) of the HIV-1 envelope glycoprotein subunit, gp120 (Fabs 59.1, 58.2, and 50.1, PDB accession numbers 1ACY, 1F58, and 1GGC). The subregion from which the peptides derive, amino acid residues 308–332, is known as the principal neutralization determinant (PND). Unfortunately, it is encoded by one of the most mutation prone sequences of the HIV genome, and the neutralizing capability of an antibody against the PND does not often generalize to mutated strains. The hope has been that structures would provide a basis for understanding how to completely neutralize the virus using antibodies, but this hope has not yet been realized. The neutralizing antibody investigated here, G3-519, recognizes an epitope from the fourth conserved region (C4) of gp120 that plays an important role in interaction with CD4 (see details below). It neutralizes various HIV-1 strains *in vitro*.<sup>1</sup> It is of interest that human hosts do not raise antibodies against this neutralization domain during infection as they do against the PND. Therefore, information about the structure of this neutralizing antibody could be of potential significance for the design of therapeutics.

**Materials and Methods.** The region of C4 to which G3-519 binds has been mapped to the peptide IIN-MWQKVGKAMYAP, residues 423–437 of gp120 from the HXB2 HIV-1 viral isolate. The antibody was produced in a cell line derived from the fusion of mouse myeloma cells, SP2/0, with antibody expressing murine B-cells. The amino acid sequences of both the light and heavy chain were determined by DNA sequencing. Crystals were grown by the hanging drop method by microseeding at room temperature from solutions at pH 5–6, 2 M ammonium sulfate, 200 mM sodium chloride, and 100 mM sodium citrate. The crystal was orthorhombic with space group P2<sub>1</sub>2<sub>1</sub>2<sub>1</sub>. Unit

cell dimensions were  $a = 37.2 \text{ \AA}$ ,  $b = 84.3 \text{ \AA}$ , and  $c = 134.0 \text{ \AA}$ . There was one molecule per asymmetric unit. The data, 90 2° frames, were processed with DENZO and SCALEPACK<sup>2</sup> for scaling and merging. The initial phase determination used the molecular replacement program X-PLOR.<sup>3</sup> The data were reprocessed with DENZO and SCALEPACK using 77 of the 90 original frames, removing weak data due to signal degradation in the final frames. Reflections extending to 2.2 Å contributed to the analysis. Starting with the preliminary structure, REFMAC<sup>4</sup> was used for the next sequence of refinement, XFIT<sup>4</sup> for real space refinement, XPLOR for simulated annealing, and CNS<sup>5</sup> for a final simulated annealing, placement of 221 waters, and bulk solvent correction. The N-terminal two amino acids of the heavy chain, glutamate-valine, proved disordered and were left out of the refinement. The final results produced  $R = 18.8\%$  and  $R_{\text{free}} = 24.7\%$ . Coordinates and structure factors have been deposited in the Protein Data Bank (1IL1).

Two programs, CE (combinatorial extension)<sup>6</sup> and TOP (automatic topological and atomic comparison program for protein structures),<sup>7</sup> were used to identify antibodies with complementarity determining regions (CDRs) that structurally resemble those of G3-519. The CDRs consist of three loops on the heavy chain and three on the light chain, all arranged to form a binding cleft at the edge of the antibody. The CE program has a requirement of a minimum of 50 contiguous amino acids in the query structure, so the variable regions of the heavy and light chains were investigated as two separate structures that were just long

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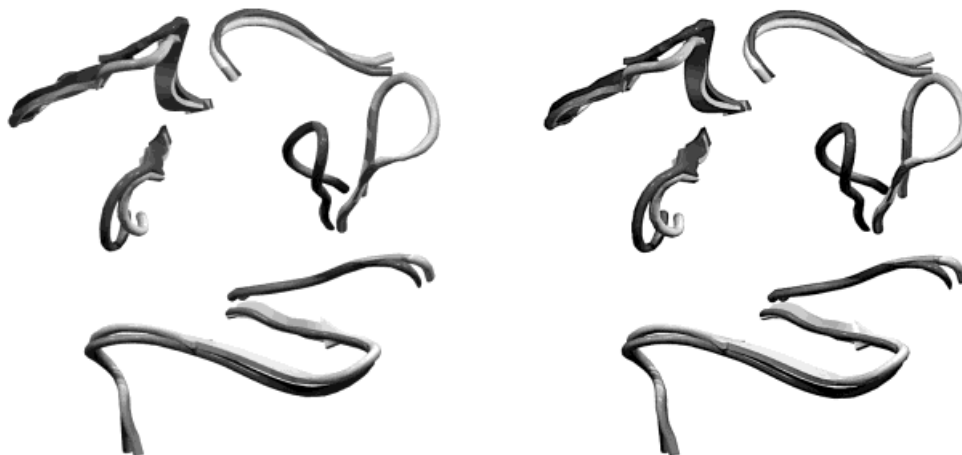


Fig. 1. Structural comparison of the CDRs of G3-519 and mCTM01. Ribbons representations of the loops were aligned by hand with use of MOLMOL and PDBview. POV-ray was used to enhance the visual depiction of the two sets of CDRs. Automated alignment merges the images of five of the six loops so well that the antibodies cannot be visualized as overlapping separate entities. The light gray ribbons represent the CDRs of G3-519, and the dark gray represents the mCTM01. The heavy chain CDR3 loops appear as the middle pair in the right side of the figure. Much of the difference between these loops is apparently due to an extra amino acid in the G3-519 loop.

enough to contain the relevant CDRs. The TOP program could match a PDB file constructed only of the six isolated CDRs, so that the barest of epitope recognition elements of the antibody could be compared to the SCOP database. Visualization of the structural relationships among similar antibodies was accomplished with MOLMOL,<sup>8</sup> PDBview,<sup>9</sup> and POV-ray.

**Results and Discussion.** The overall structure is similar to that of other Fab fragments whose three-dimensional structures have been solved. When both heavy chain and light chain CDRs were considered, TOP and CE identified the antitumor antibody, mCTM01 (Protein Data Bank accession number 1AE6),<sup>10</sup> as most similar. The antibody mCTM01 was raised against MUC1, a mucin overexpressed on the surface of several cancer cell types. The likely antigen for this antibody is the glycosylated tandem repeating peptide sequence of the mucin, AHGVT-SAPDTRPAPGSTAPP. There is little similarity between this sequence and that of the C4 peptide used to generate G3-519. However, the positions of the C-alpha carbons in the binding cleft CDRs of both antibodies are quite similar except for those of the heavy chain CDR3 (Fig. 1) and the sequences of the CDRs of both antibodies are similar. The significance of these similarities, if any, is not clear. To our knowledge, no studies of cross-reactivity between G3-519 and mCTM01 have been performed.

The binding of gp120 to CD4 on the T-cell surface is at least partially mediated by the C4 region of gp120. One of the C4 peptide sequences involved in this binding is NMWQKV, corresponding to the third through eighth amino acids of the C4 peptide to which the G3-519 immunogenic epitope has been mapped. CD4-gp120 binding also alters the conformation of the gp120 region that binds chemokine receptor CCR5. This alteration is apparently necessary for gp120-CCR5 binding as well as for binding of the antibody 17b.<sup>11</sup> The possibility arises,

therefore, that, on binding, an antibody to the C4 region of gp120 (e.g., G3-519) may alter the presentation of epitopes in the relatively conserved CCR5 binding regions of gp120. Specifically, it appears that a refined version of G3-519 would not only block CD4 association but also expose other segments of gp120 to another antibody, thereby creating the opportunity for dual antibody passive immunity.

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