Fab FRAGMENTS OF AN ANTI V3 MONOCLONAL ANTIBODY NEUTRALIZE HIV-1

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INTRODUCTION

Passive immunotherapy with monoclonal antibodies (Mabs) is one of the many approaches that are presently been followed in the fight against AIDS. The V3 loop of gp120, as the immunodominant neutralizing domain has been the main target for these purposes (1). Protection after passive infusion of anti V3 Mabs has been reported in chimpanzees and SCID mice (2, 3). We have previously developed two neutralizing murine Mabs against the V3 region of the MN strain (4). In the present work we described the fine mapping of the epitope recognized by these Mabs and demonstrated that Fab fragments conserve its neutralizing potential.

EXPERIMENTAL PROCEDURES

Pure preparations of Mabs 2C4 and 10F10 were obtained from ascitic fluid by Protein A chromatography. The fine mapping of the epitope was carried out using a set of 12-mer alanine substituted peptides. Two ELISA procedures were used: 1) indirect ELISA with each peptide coupled to polystyrene plates and 2) Competitive ELISA with incubation of peptides and Mabs in solution. Fab fragments of Mab 10F10 were generated after one hour incubation with immobilized papain, and purified by HPLC chromatography on a TSK2000 column. The activity of the fragment was monitored by ELISA, and the neutralizing capacity was measured in a classic assay with the MN strain using MT4 cells. The RNA was extracted and used to generate cDNA. The V regions were PCR amplified using degenerate oligonucleotides for signal peptide and constant region for both heavy and light chains. The fragments were cloned and sequenced.

RESULTS AND DISCUSSION

In this work we restricted the minimal length of the epitope recognized by Mabs 2C4 and 10F10 to the 12-mer peptide KRIHIGPGRAFY. The fine mapping of this epitope revealed that there was only one subtle difference between both Mabs. While substitution of proline in position 7 completely abolished the binding of 2C4, only reduced in 50% the binding of 10F10. This difference should respond to some of the amino acid changes that we found in the V region sequences.

The Fab fragments of Mab 10F10 neutralized the MN strain of HIV-1, as shown in figure 1.

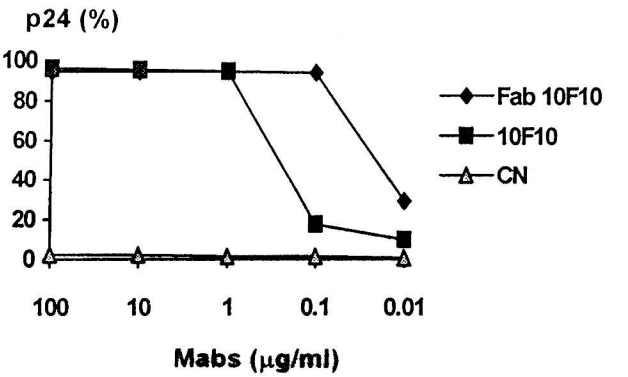

Fig. 1 Neutralization of HIV-1 (MN). I=% inhibition P24

The fact that virus neutralization by anti V3 Mabs is a Fc independent process, favours the use of Fab or Fv fragments produced in bacteria for immunotherapy. We believe that a cocktail of these reagents directed against different HIV-1 clades could be a beneficial therapy for HIV-infected persons.

REFERENCES

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