THE ANTIBODY RESPONSE OF MACAQUES IMMUNIZED WITH THE MULTI-EPITOPE POLYPEPTIDE TAB9 IN MONTANIDE ISA 720

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Introduction

The third variable region (V3 loop) of the HIV external glycoprotein gp120, is considered to play a key role in viral infectivity, tropism, and syncytium formation (1). This segment contains the Principal Neutralizing Domain of the virus (2) bearing sequential B cells epitopes as well as epitopes for T-helper and T-cytotoxic cells. Passive transfer of monoclonal antibodies against V3 can protect against viral challenge in SCID mice (3) and chimpanzees (4). Anti-V3 titers correlate with protection in immunized chimpanzees and are inversely related to disease progression in HIV-1 infected people (5). These experimental evidences suggest that a vaccine able to elicit high anti-V3 titers could confer protection against HIV-1 infection.

Our group has reported the generation of Multi-Epitope Polypeptides (MEPs) expressing the V3 region of several HIV-1 isolates "in tandem" fused to a stabilizing sequence as a novel alternative to generate a wide antibody response against V3. In the present work we analyzed the humoral response elicited in macaques after immunization with one of these MEPs: TAB9 adjuvated in Montanide ISA720.

Materials and Methods

The MEP TAB9 was expressed at high level in *Escherichia coli* and purified to more than 95.4 % using chromatographic procedures according to Good Manufacture Practices (GMP). Two groups of macaques (n=4) were immunized with four intramuscular inoculations of 1mg or 200 µg of TAB9 in Montanide ISA720 on days 0, 30, 180 and 360. Animals were bled on days 45, 180, 210, 270, 360 and 390. The time course of anti-TAB9 antibody response was evaluated by ELISA. Microtiter plates (High Binding, Costar, SA) were coated with 2 µg/mL of either the recombinant protein or BSA coupled peptides. Serial dilutions of the sera were evaluated with TAB9 and a single dilution (1:100) was assayed against peptides. The anti-monkey Ig Horseradish peroxidase conjugate was used as a second antibody. All sera were analyzed in duplicate. Cut off values were calculated for each plate as twice the optical density obtained for negative sera. The last dilution that gave absorbance values higher than the cut off was regarded as the serum titer. The results obtained were processed using the StatWin program.

Results and Discussion

The sera from the immunized animals were tested in ELISA for antibody reactivity against the protein TAB9. Specific antibodies appeared after the first inoculation in all macaques. A strong anamnestic response was detected after the second injection. A similar behavior was observed after the third and fourth injections (Figure 1). The time course of anti-TAB9 antibody response was very similar for the two doses employed. There were not statistically significant differences ($p>0.05$) between the geometric means of the titers in both groups.

The reactivity against homologous and heterologous peptides representing Cuban HIV-1 isolates and cladistic consensus was assayed after three inoculations. A strong antibody response with more than 90 % of reactivity against the V3 peptides included in the protein were developed in both groups. The antibodies generated by immunization with TAB9 recognized, with high or medium reactivity, peptides representing 62 % of the Cuban isolates analyzed. A high degree of cross-reactivity against cladistic consensus peptides, representative of the viral subtypes circulating worldwide was observed, even when clade B was the only well represented in the immunogen.

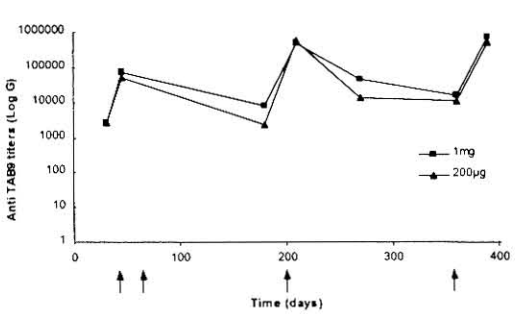

Figure 1: Time course of anti TAB9 antibody response in macaques using two different doses.

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