present only in TAB13, did not increase when this protein was used as immunogen (Table 2).

From this set of experiments we concluded that the inclusion of two V3 regions reduced the immunogenicity of the MEP in mice and rabbits.

As the molecular weight of TAB13 is only 1.2 times higher than TAB9 we do not consider that the corresponding differences in the number of molecules from each proteins were relevant for the immunogenicity. Another explanation that could be advanced is the existence of a differential surface exposure of the V3 epitopes between MEPs. To address this problem we analysed the binding of equimolar amounts of both proteins in a two site sandwich ELISA using specific MAbs.

Four MAbs against the V3 regions MN, LAI and JY1 were used as capture antibody. HRP-conjugated MAb 448, directed against the p64K N terminal fragment was used as second antibody. From this experiment we concluded that the average of the absorbance values for 10 replicated samples were always significantly higher for TAB9. These differences, although moderate, were consistent in several experiments indicating a preferential surface exposition of the V3 epitopes in TAB9. It seems hard to conclude that these modest differences are the responsible for the reduced immunogenicity of TAB13 but it is attractive to think that it could at least influence on it. It is possible to speculate that as the molecular weight increase the intramolecular interactions augments, and the protein acquired a more complex folding pattern and globular structure affecting the accessibility of the V3 regions and therefore the immunogenicity.

If the number of V3 to be included in a MEP is limited by these adverse effects it would be necessary the adequate selection of the V3 sequence to be include in the MEP in order to cover as many HIV-1 variants as possible. An alternative approach could be the design of immunogens by geographic area based on the data of the molecular epidemiology of HIV-1 and specifically the V3 region.

NOVEL AFFINITY SITE FOR ANTIBODY MEDIATED DIAGNOSTIC AND THERAPEUTIC TARGETING

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The recent discovery (PNAS 93:6019-6024, 1996) of a novel affinity site on antibodies offers a new methodology to couple to antibodies therapeutically active and radio-image producing compounds.

Furthermore, this new affinity linking technology allows the chemical attachment of reporter molecules for immuno-assays and in situ diagnostic. Currently applied coupling methods attach randomly active compounds to the antibody surface which can alter or block the sensitive antibody binding site. The new method takes advantage of a naturally existing site which has high affinity for heterocyclic structures such as ATP and Adenosine. The phosphate group and the ribose of both compounds can be used to attach chelates for tumor imaging and radio-immunotherapy, oligonucleotides for antisense gene therapy and reporter molecules for immunodetection assays.

For example, biotin was conjugated to Azido-ATP via a phosphate ester linkage and to the oxidized ribose of azido-adenosine via aldehyde condensation reaction. Photo-affinity biotinylated antibodies showed excellent signal-to-noise ratios in ELISA, FACS and Western blots. Furthermore tumor imaging with photo-affinity-chelated antibodies produced high uptake of isotope labeled antibodies by tumors in an animal model. Finally, this new affinity linker technology can be performed on antibody containing solutions without prior and post reaction purification steps.