THE HUMORAL IMMUNE RESPONSE OF RABBITS AND MICE AGAINST MULTI-EPITOPE POLYPEPTIDES ARRYING DIFFERENT V3 REGIONS OF HIV-1

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Introduction

The V3 loop of the gp120 contains the Principal Neutralizing Domain of HIV-1 (1). Passive transfer of monoclonal antibodies (MAbs) against this region protects chimpanzees and SCID mice from viral challenge (2, 3), and correlates with protection in immunized chimpanzees (4), suggesting that they may be important to confer protection in humans. We have constructed eleven different variants of multi-epitope polypeptides (MEPs) expressing the V3 region from different HIV-1 isolates, as a novel approach to induce a broadly reactive antibody response against a variable region (5, 6). In this presentation we present comparative data on the humoral immune response against two of these proteins: MEPs TAB9 and TAB13. The differences in the immunodominance of the V3 epitopes in rabbit and mice is also highlighted.

Materials and Methods

A DNA fragment encoding for 15 amino acids regions of the V3 loop from HIV-1 isolates LR150, JY1, MN, RF, BRVA, and LAI was previously synthesized and cloned fused to the amino terminal fragment of the human IL2 in the plasmid named pTab4 (6). The V3 regions are linked by the spacer AGGGA. The V3 regions included in TAB4 were selected from the V3 sequences reported by La Rosa, *et al.* (7) according to two basic criteria: (a) the fifteen amino acid central region of V3 contains the neutralizing epitopes. (b) two isolates will group together if they share at least 7 straight amino acid.

The central tetrapeptide of the V3 loop is the more conserved sequence in this region and forms a type II Beta turn. Five of the selected V3 regions contains the central motive GPGR, which is typical for the subtype B circulating meanly in Europe and the Americas. The other region (JY1) contains the motive GLGQ which has been reported in viruses from subtype D, circulating in Africa and Asia (Table 1).

The IL2 coding region in pTab4 was then replaced by a DNA fragment encoding for the 44 amino acid amino terminal part of the P64K protein of *Neisseria meningitidis*. The resulting plasmid was named pTab9 and encodes for a fusion protein of 17 kD that was called TAB9. TAB13 was derived from TAB9 through the insertion of two additional V3 regions, C4-11 and C4-13 before the C terminal epitope (LAI), and has a molecular weigh of 20 kD.

Both proteins were expressed as inclusion bodies in *Escherichia coli*, readily purified and administered to mice and rabbits using different adyuvants. The MEP format was compared with the more classical approach of chemical conjugation by immunising mice with TAB9 and a mixture of the six V3 synthetic peptides coupled to bovine seroalbumin (BSA).

Results and Discussion

MEP TAB9 was more immunogenic in mice than BSA conjugated peptides

First we compared the immune response of Balb/C mice against TAB9 and a mixture of V3 synthetic peptides coupled to BSA. The amount of TAB9 and BSA-V3 peptides were adjusted to approximately the same nolarity of V3 epitopes. Specific antibodies against proteins and V3 peptides were measured by an indirect ELISA. After two intraperitoneal (IP) injections in Freund's Complete Adjuvant a strong positive signal against JY1 was observed in the TAB9 group, together with a weak but positive reaction against peptides BRVA and MN. In contrast no response against any of these peptides was observed in the V3-BSA immunized group. The rest of the peptides tested (LAI, LR150 and RF) were immunosilent in both immunogens.

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3. Safrit J *et al.* AIDS 1993;7:15-21.

4. Berman PW *et al.* Nature 1990; 345: 622-625.

5. Duarte CA *et al.* AIDS Res Hum Retroviruses 1994;10(3):235-243.

6. Montero M *et al.* Submitted to Vaccine.

7. La Rosa GJ *et al.* Science 1990; 249: 932-935.

Table 1. V3 epitopes included in MEPs.

Strain	Sequence	Clade
LR150	SRGIRIGPGRAILAT	B
BRVA	RKRITMGPGRVYYTT	B
RF	RKSITKGPGRVIYAT	B
IIIB	SIRIQRGPGRAFVTI	B
JY1	RQSTPIGLGQALYTT	D
MN	RKRIHIGPGRAFYTT	B
C4-11	TSITIGPGQVFYRTG	
C4-13	RQRTSIGQGQALYTT	

To explain this experimental observations we can advanced three possibilities: (a) the P64 moiety in TAB9 provided more efficient T cell help than BSA, (b) the linkage of the six V3 regions in the same molecule provide efficient T cell help among each other, (c) the antibody response is diverted by immunodominant and more abundant B cell epitopes in the BSA.

Immunodominance among V3 regions and the influence of the H-2 haplotype

To investigate the influence of the H-2 haplotype in the antibody response against TAB9 and the V3 regions we immunized mice from three different haplotypes. Haplotype H2d, which includes Balb/C, showed a pattern of reactivity similar to the one described. However, mice from haplotypes H-2k of H-2b essentially do not respond to any of the V3 peptides or even against TAB9. In the few sera where some response could be detected these were also directed against JY1, LR150 or MN.

Since our main goal is to generated a wide antibody response against V3 from different HIV-1 subgroups, the relationship of immunodominance among V3 peptides having different but related amino acid sequence is of primary importance. From the experiments done in mice it was clear that the immunodominance of V3 regions follows the order: JY1>MN, BRVA >LAI, LR150, RF. The three last regions were practically immunosilent in our experiments.

Taking into consideration these results, we hypothesized that the regulation of the immunological repertoire in mice account for the restriction of the antibody response to certain V3 sequences. Looking for patterns of homology we made the interesting observation that the GPG sequence was present five times, regularly spaced along the V Beta chains of the TCR in mice. We had previously shown that JY1 peptide, the more immunogenic region in TAB9, is the only one which lack the motif GPG in the central part of the loop. This findings lead us to propose that B cells expressing surface receptors for this motif are submitted to a more rigorous control by the idiotipic network in mice.

In the rabbit model, where the V3 region is more immunogenic, both MEPs elicited high antibody titers against the protein. Additionally this response in rabbits was more evenly distributed among V3 peptides. Some of these sera also showed neutralizing activity against HIV-1 laboratory isolates. These results confirm that the animal species have a major influence in the antibody response against V3.

Comparison of the immunogenicity of TAB9 and TAB13

Previous studies in rabbits of the immunogenicity of other MEPs containing the same six V3 regions showed a high degree of cross-reactivity against V3 peptides with the central regions GPGR or GLGQ, homologous to those present in the immunogen. However 1 or 2 orders lower antibody titers were found against other peptides with the central region GQGR and GQGQ circulating in Africa and Asia. We incorporated these peptides in TAB13 to generated a stronger antibody reponse against these sequences.

However, when the immunogenicity of TAB9 and TAB13 was compared we found that the geometric mean of the antibody titers against protein was one order of magnitude higher in mice immunized twice with 10 μg of TAB9 than in mice immunized with four different batches of TAB13. Interestingly, equivalent titers were observed when the amount of TAB13 was doubled up to 20 μg. When we next looked at the time course of the antibody response against the proteins in rabbits we found a similar effect.

Finally, either the geometric mean of the antibody titers against peptides or the frequency of response against V3 peptides were significantly higher for the TAB9 group compared with TAB13. Even antibody titers against peptides C4-11 and C4-13, that are

Table 2. Antibody responses against V3 in rabbits immunized with TAB9 and TAB13.

Immunogens	LR150	JY1	RF	MN	BRVA	IIIB	G	F	C4-11	C4-13
TAB9	51200	204800	102400	204800	51200	102400	102400		51200	6400
	25600	25600	6400	102400	6400	102400	25600		< 100	< 100
	1600	51200	12800	6400	3200	. 400	4525		3200	6400
	204800	12800	51200	102400	204800	6400	51200		3200	102400
G	25600	43054	25600	60887	21527	12800	27917	100	8063	16127
TAB13	< 100	12800	< 100	3200	12800	3200	6400		6400	< 100
	25600	204800	51200	102400	51200	12800	51200		51200	6400
	25600	6400	< 100	3200	< 100	< 100	8063		< 100	< 100
	1600	6400	. 400	6400	< 100	12800	3200		6400	< 100
G	10159	18102	4525	9051	25600	8063	10973	75	12800	6400

G: Geometric mean of antibody titers.
F:Frequency of response against V3 peptides.

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 al ways 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.