Research of phage display-selected peptides with specific affinity for Vascular Cell Adhesion Molecule-1 (VCAM-1) overexpressed in atherosclerotic plaques

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INTRODUCTION

➢ **Acute atherothrombotic syndromes** (i.e. myocardial infarction, brain stroke etc.) represent the leading cause of morbidity and mortality in the developed countries.

➢ Despite major advances in the treatment of coronary heart disease, a large number of the disease's victims presenting an apparently healthy constitution die suddenly without prior symptoms (Figure 1).

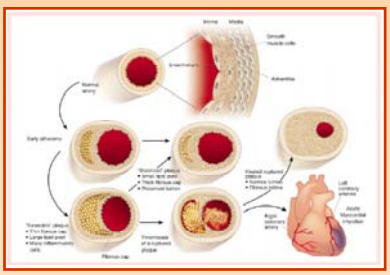


Figure 1. Schematic representation of the atheroma's evolution [1].

➢ **VCAM-1**, overexpressed in inflammatory conditions, is expressed by endothelial cells (Ecs) and by smooth muscle cells (SMCs) of the diseased artery itself and of the microvascular network of vasa vasorum in atherosclerotic plaques.

➢ Neovascularisation and expression of adhesion molecules by microvessels at sites of vulnerable lipid-rich plaques could contribute to **plaque destabilization**.

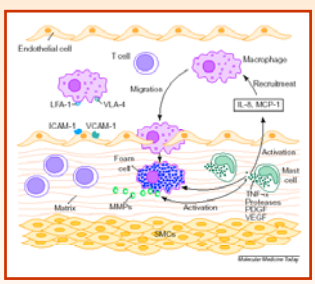


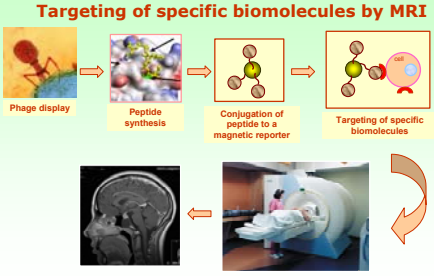
Figure 2. Inflammatory mechanisms in atherosclerosis [2].

PURPOSE OF THE WORK:

➢ To screen by **phage display** for VCAM-1 peptide binders with the final purpose to diagnose vulnerable atherosclerotic plaques by MRI after peptide conjugation to a paramagnetic or superparamagnetic contrastophore (magnetic reporter) (Figure 3).

Figure 3. Phage display:

Targeting of specific biomolecules by MRI



MATERIAL AND METHODS

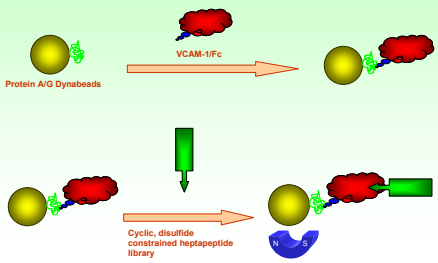
➢ The **phage display screening** (4 rounds) performed *in vitro* (Figure 4):

➢ **disulfide-constrained heptapeptide library** (Peptide Library Kit, New England BioLabs® Inc., Westburg b.v., Leusden, The Netherlands).

➢ **the target :** recombinant mouse VCAM-1/Fc Chimera (R&D Systems Europe Ltd., Abington, UK) immobilized on magnetic nanoparticles Dynabeads® Protein A/G (Dynal Biotech, Compiègne, France).

Figure 4. Panning:

VCAM-1 immobilized on magnetic nanoparticles



➢ **42 phage clones** isolated and characterized.

➢ **The affinity** for human (recombinant or expressed by HUVECs) and mouse VCAM-1 was **evaluated**

➢ **Competition experiments** (VCAM-1 in solution or VLA-4 expressing cells) confirmed the specific interaction with VCAM-1

➢ The **DNA was sequenced** (dideoxynucleotide method of Sanger) and peptide structure was translated

➢ **4 peptides were selected** for further characterization

➢ The **biotinylated peptides** were synthesized (NeoMPS, Strasbourg, France) and their **affinity constants** were evaluated

➢ The **binding to atherosclerotic plaques** was evaluated by immunohistochemistry on aorta specimens harvested from ApoE mice.

Figure 8. Biotinylated R831 and R832, anti-biotin antibody, HRP-secondary antibody, DAB, Hemalum, Luxol fast-blue, 10x

ApoE$^{-/-}$ mouse (15 month old, fed on cholesterol diet)

VCAM-1 expression was confirmed with rabbit polyclonal anti-VCAM-1 antibody (Santa Cruz, Heidelberg, Germany), followed by biotinylated anti-rabbit antibody (Vector Labconsult, Bruxelles, Belgium)

Black flashes indicate the endothelium
Red flash indicates media (smooth muscle cells)

Negative control (no peptide)

CONCLUSIONS

➢ The *in vitro* evaluation of this peptide pleads for a **specific interaction with the targeted biomolecule**.

➢ The conjugation of R832 to magnetic reporters could help at the **diagnosis of atherosclerotic disease**, both during its precocious stages and later, when the plaque is prone to rupture and thrombosis.

REFERENCES

[1] Libby P, Nature 420 (2002) 868.

[2] Kelley J et al, Molecular Medicine Today, 6, 2000, 304.

RESULTS

➢ The 42 phage clones isolated after four rounds of biopanning present an **important affinity both for mouse and human VCAM-1** (Figure 5).

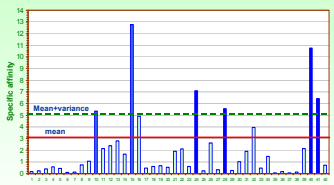
➢ **The sequences** presenting the amino acids T, R and L were enriched after four rounds of panning (Figure 6).

➢ **Peptide alignment** with adhesion molecules (integrin, protocadherin) or with immunoglobulin receptors shows that their selection was not accidental (Table 1).

➢ Based **on K^*_d and IC^*_{50} values**, peptide expressed by phage clone 40 (R832) was selected for subsequent *in vitro* and *in vivo* evaluation (Figure 7).

➢ The binding of peptides R831 and R832 to atherosclerotic plaques was confirmed by immunohistochemistry (Figure 8).

Figure 5. Coefficient of specific affinity (SA) for VCAM-1 of 42 phage clones selected after 4 rounds of panning

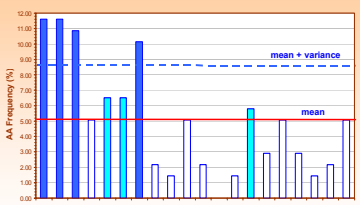


$$SA \sim \left[\left(\frac{hVCAM1_{ass} \times mVCAM1_{ass} \times HUVEC_{ass}}{hVCAM1_{ass}}\right)^2 \Big/ \sqrt{Jurkat_{ass} \times Jurkat_{dis}}\right] \times 100$$

HUVEC = human umbilical vein endothelial cells

-hVCAM1 = affinity for human recombinant VCAM-1
-mVCAM1 = affinity for mouse recombinant VCAM-1
-HUVEC = affinity for HUVEC+TNFα
-hVCAM1$_{ass}$ = pre-incubation with human VCAM-1
-Jurkat$_{ass}$ = post-incubation with Jurkat
-Jurkat$_{dis}$ = pre-incubation with Jurkat

Figure 6. Amino acid frequency in the peptide structure



3 first positions (N-terminal): Ser, Thr,
4 last positions (C-terminal): Arg, His, Thr

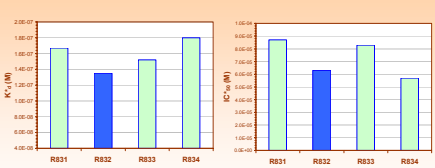
⇒ ionic or hydrogen interactions with VCAM-1

Table 1. Alignement (BLAST) of selected peptides with the sequence of known proteins

Clone	Peptide alignment
10	Low affinity IgE Fc receptor isoform A and C
	Fc epsilon receptor II
	Similitude with α-4 subunit of VLA 4
22	T-cell receptor delta chain
40	Leukocyte Ig-like receptor B
	Leukocyte common antigen-related protein
	Protocadherin
41	Integrin alpha 2b
	Leukocyte Ig-like receptor A
	Similitude with α-4 subunit of VLA 4

Figure 7. K^*_d and IC^*_{50} values for the interaction of peptides with VCAM-1

IC^*_{50} values were evaluated in competition with Jurkat T cells



Biotinylated peptides:
Clone 41 = R831
Clone 40 = R832
Clone 22 = R833
Clone 10 = R834