Molecular imaging of VCAM-1 expression in inflammatory pathologies by using low-molecular weight peptides conjugated to a paramagnetic reporter

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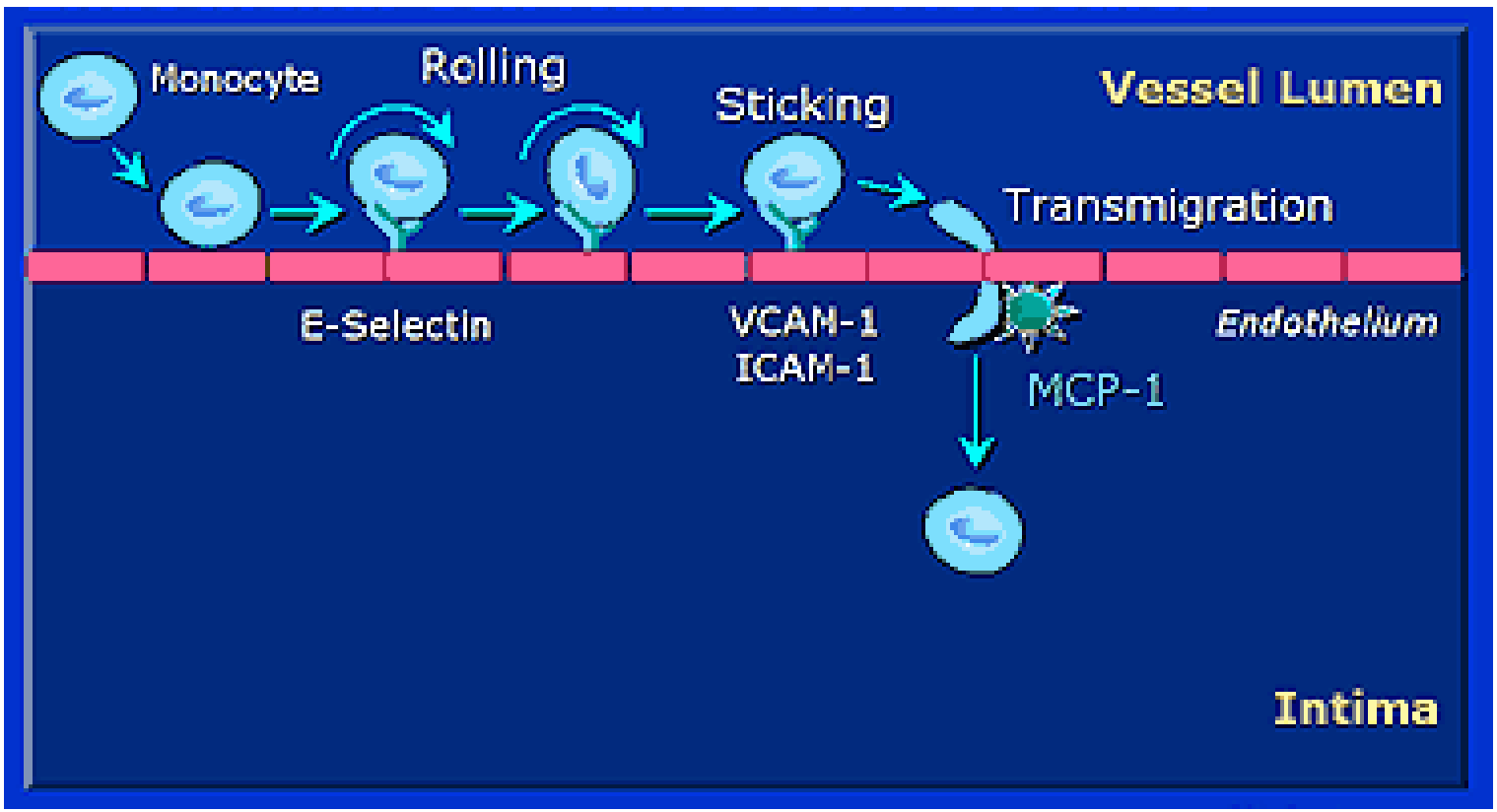
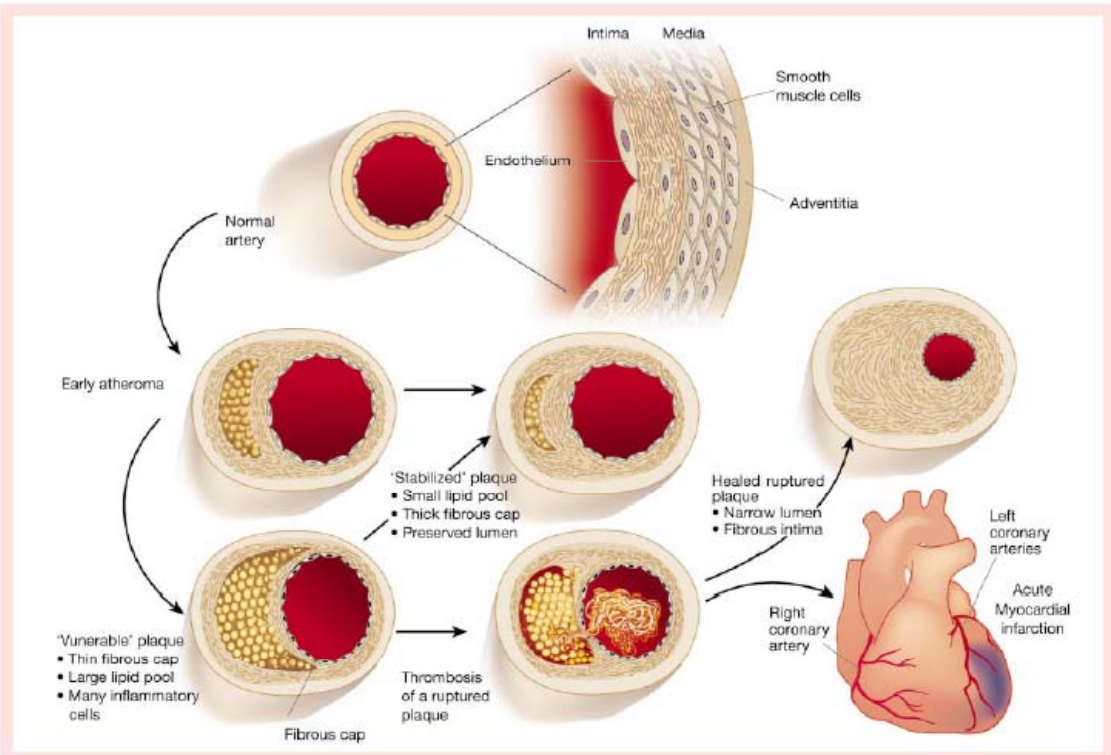
Inflammation:

Recruitment of leukocytes by endothelial cell adhesion molecules



Charo IF, Curr Opin Lipidol, 3, 1992, 335

Cardiovascular diseases: complications of atherosclerosis

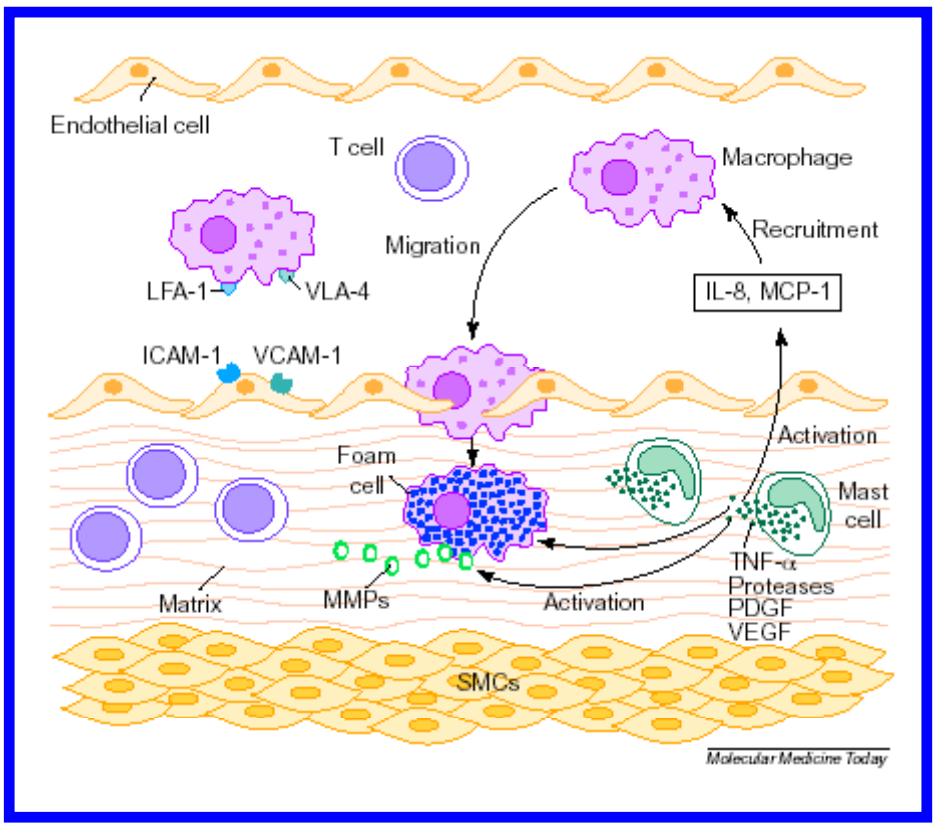


Libby P, Nature, 420, 2002, 868 – 874

- Important progress in the therapy and prevention of cardiovascular diseases

- Still, myocardial infarction and brain stroke ➔ the main causes of death in Occidental countries

Inflammation mechanisms in atherosclerosis



- Lesion of arterial wall by risk factors:
  - hypercholesterolemia, diabetes, smoking and hypertension
- Inflammatory response:
  - Expression of adhesion molecules
  - Synthesis of cytokines and growth factors
  - Endothelial adhesion and transmigration of leukocytes
  - Monocytes → macrophages → foam cells
  - Migration of smooth muscle cells (SMC) from media to intima
  - Collagen synthesis

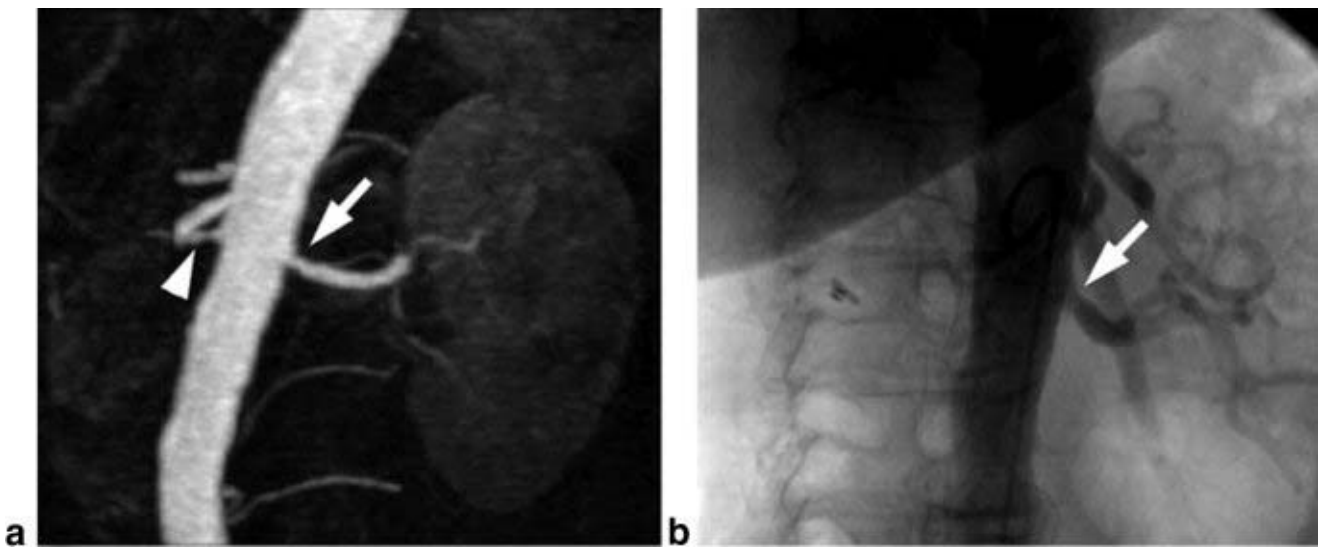
Kelley J et al, Molecular Medicine Today, 6, 2000, 304

Classical/current diagnosis of atherosclerosis

- Evaluation of arterial stenosis: MRA, Doppler ultrasonography, intra-arterial digital subtraction angiography

Schneider G et al

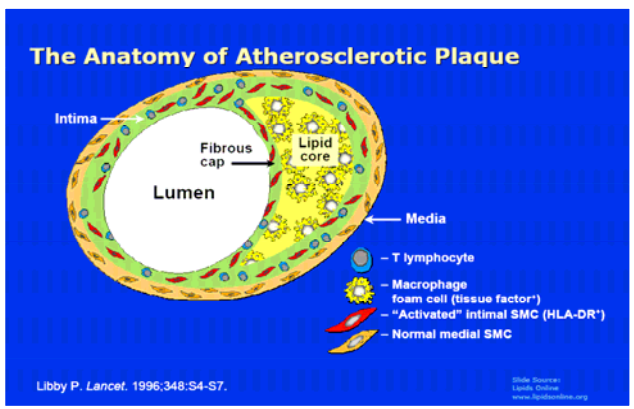
J Magn Reson Imaging, 26, 2007, 1020

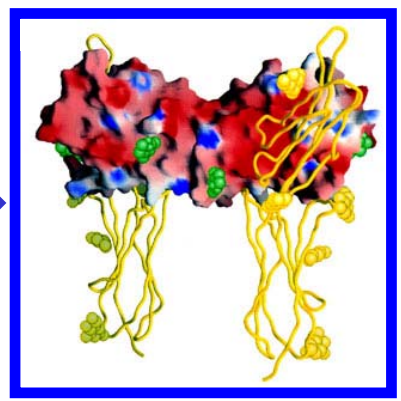


CE-MRA (a) reveals moderate ostial stenosis (arrow) of the left renal artery. The right renal artery (arrowhead) is hypoplastic. The DSA image (b) confirms the diagnosis of ostial stenosis (arrow) of the left renal artery as demonstrated on CE-MRA.

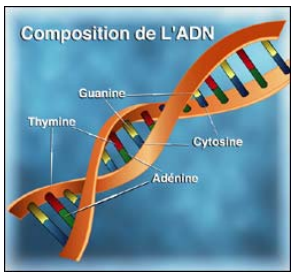
Specific diagnosis of atherosclerosis

- Atherosclerosis is often asymptomatic
- Plaque rupture without stenosis
- Need to specifically diagnose vulnerable atherosclerotic plaques ➔ detection of characteristic biomolecules ➔ molecular imaging

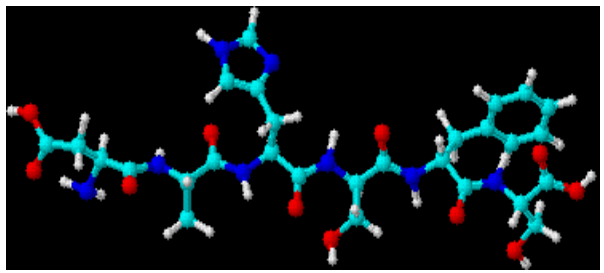




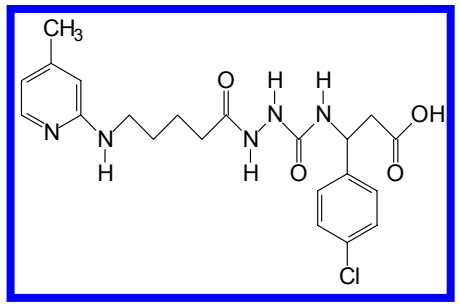
Tracers for magnetic resonance molecular imaging



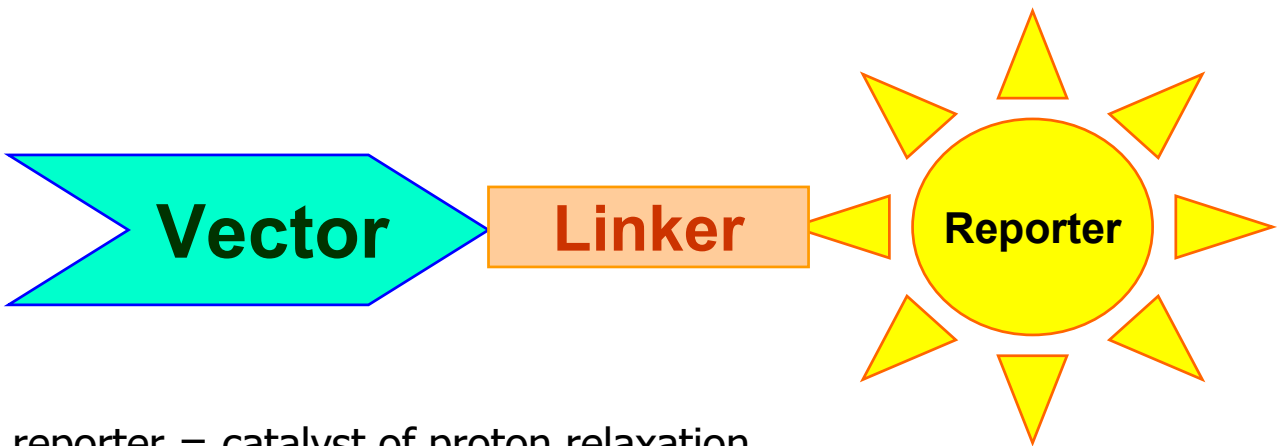
Oligonucleotides (SELEX)



Peptides (phage display)

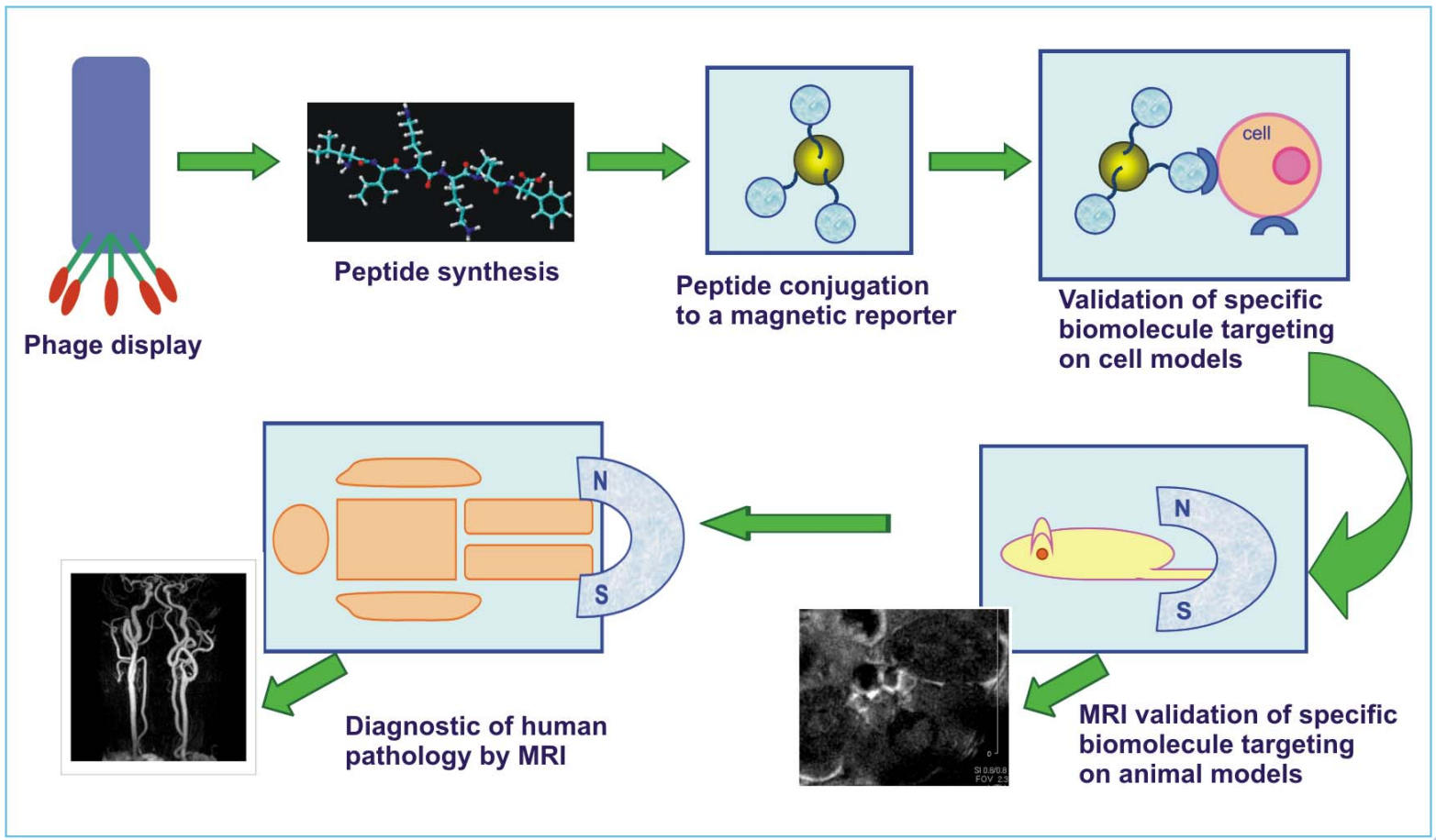


Organic molecules of synthesis

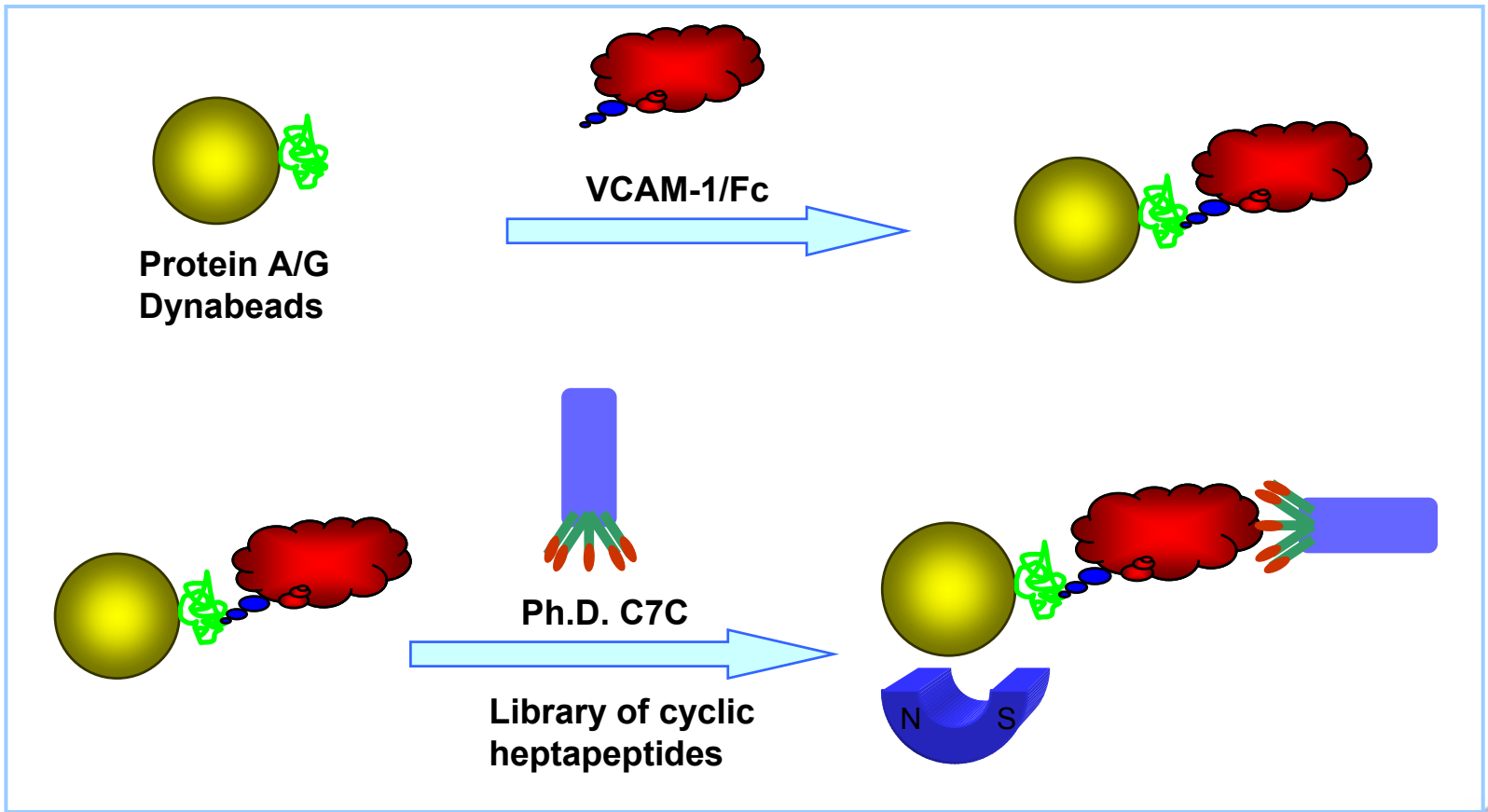


- MRI reporter = catalyst of proton relaxation
  - High relaxivity and magnetic susceptibility ➔ detection in areas of low target concentration

Peptide vectorized contrast agents for molecular imaging

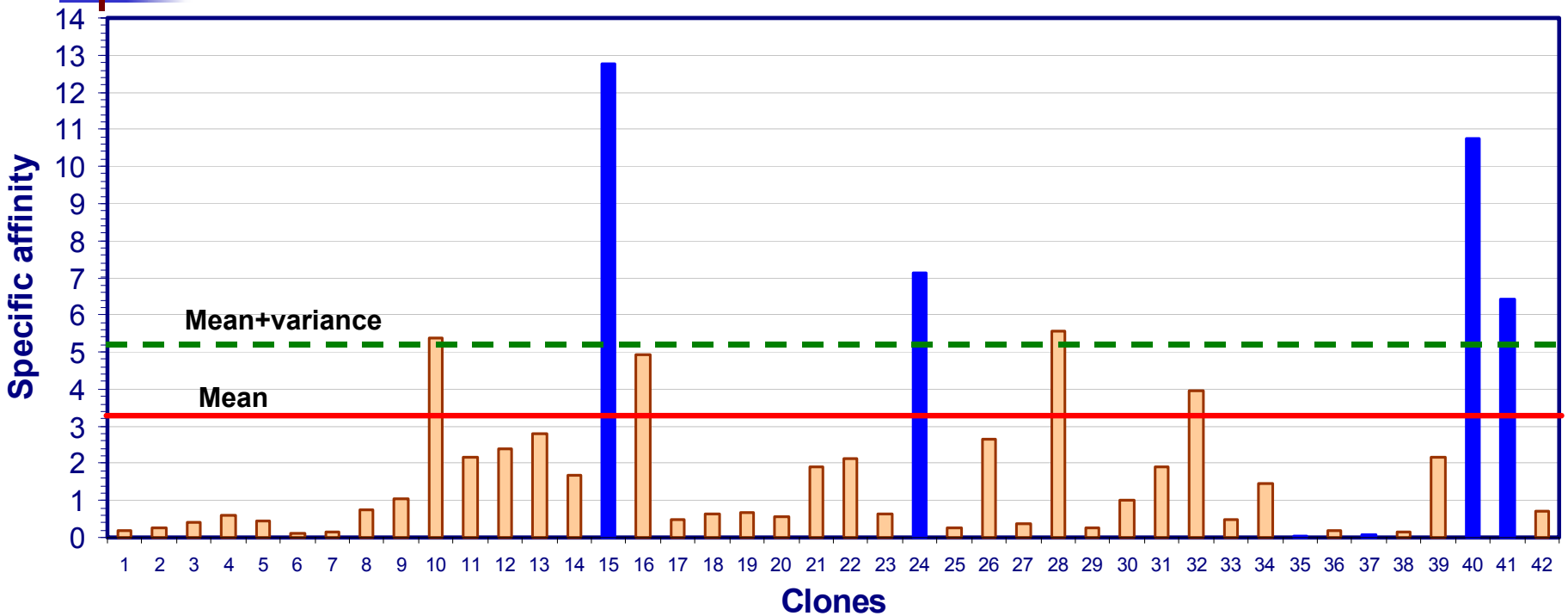


Phage display: Selecting VCAM-1 specific peptides



Clones selected after 4 rounds of panning

Coefficient of specific affinity for VCAM-1

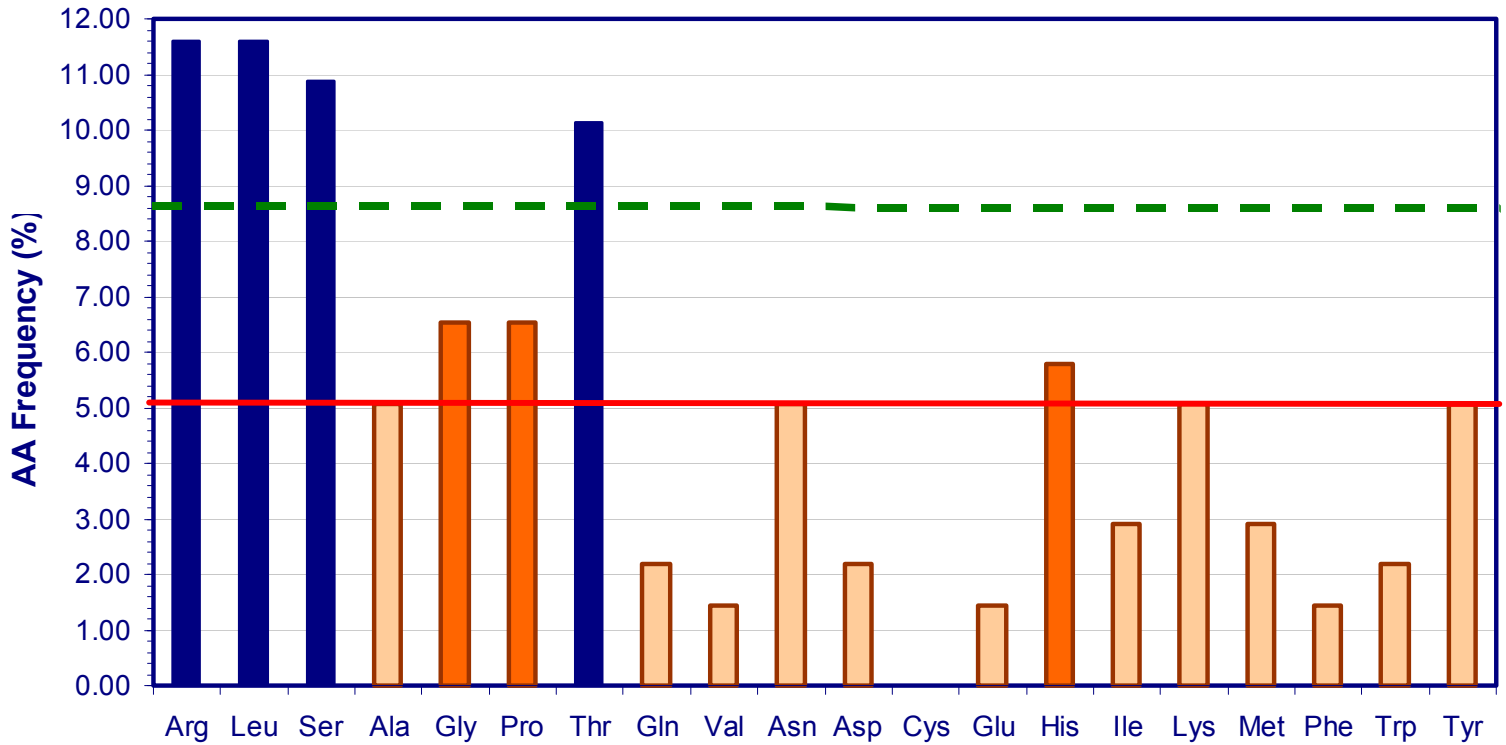


$$SA = \left[\left(\frac{hVCAM1 \times mVCAM1 \times HUVEC}{hVCAM1_{pre}} \right)^2 \Big/ \sqrt{\left(Jurkat_{post} \times Jurkat_{pre} \right)} \right] \times 100$$

-hVCAM1 = affinity for human recombinant VCAM-1
-mVCAM1 = affinity for mouse recombinant VCAM-1
-HUVEC = affinity for HUVEC+TNFα
-$hVCAM1_{pre}$ = pre-incubation with human VCAM-1
-$Jurkat_{post}$ = post-incubation with Jurkat
-$Jurkat_{pre}$ = pre-incubation with Jurkat

HUVEC = human umbilical vein endothelial cells

Amino acid frequency in the peptide structure

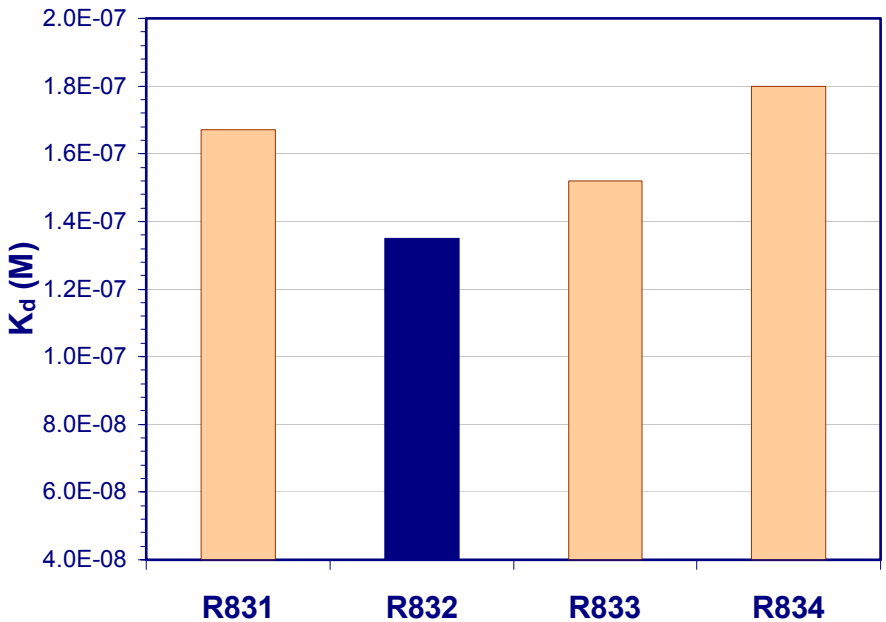


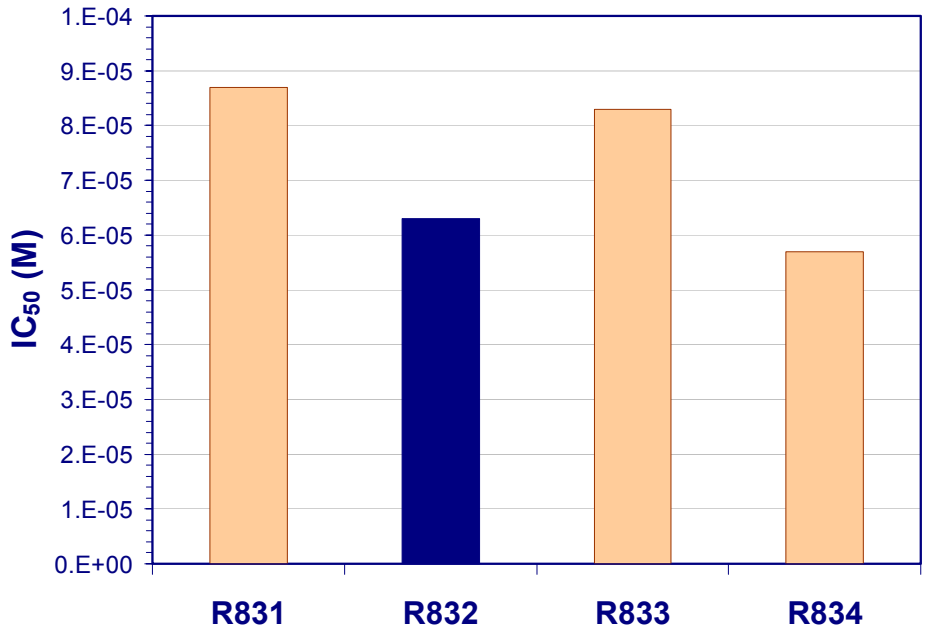
Arg, His, Ser, Thr ➔ ionic or hydrogen interaction with VCAM-1

Peptide homology (BLAST) with sequence of relevant proteins

Peptide symbol	Peptide alignement
R834	α-4 subunit of VLA 4
R833	T-cell receptor delta chain
R832	Leukocyte Ig-like receptor B Leukocyte common antigen-related protein Protocadherin
R831	Integrin alpha 2b Leukocyte Ig-like receptor A α-4 subunit of VLA 4

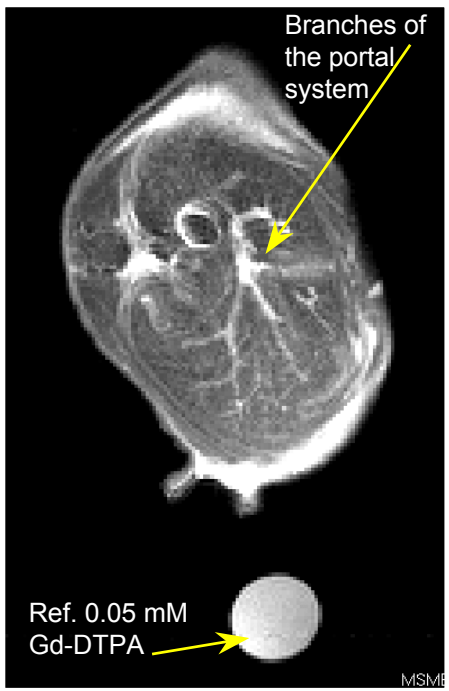
K_d estimated for the interaction with VCAM-1
IC_{50} estimated in competition with Jurkat T lymphocytes



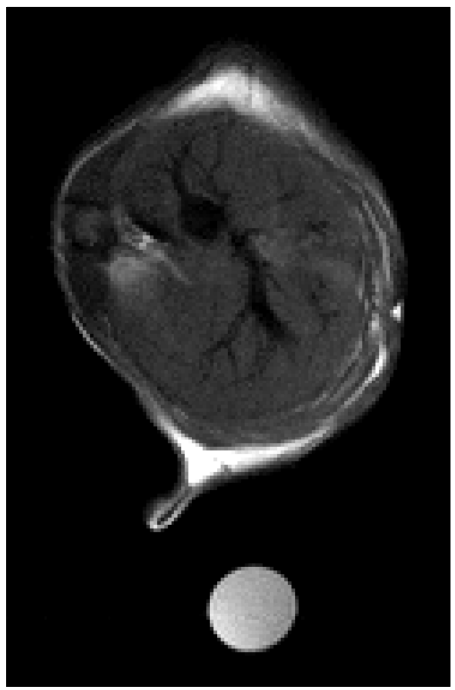


Molecular MRI of VCAM-1 expression in a mouse model of hepatitis (concanavalin A) with Gd-DOTA-R832 (1h30' post-contrast)

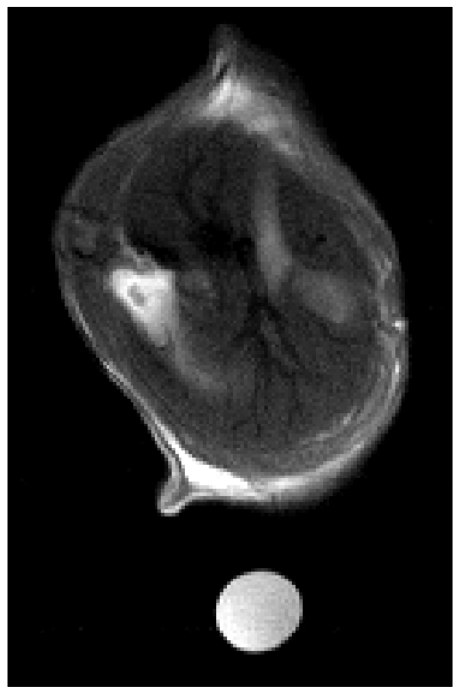
Avance-200 MRI, 4.7 T, MSME (TR/TE = 307.4/14.7 ms, FOV = 5 cm, slice thickness = 3 mm, matrix = 256, NEX = 4, TA = 5'14" spatial resolution = 195 µm)



Hepatitis Gd-DOTA-R832

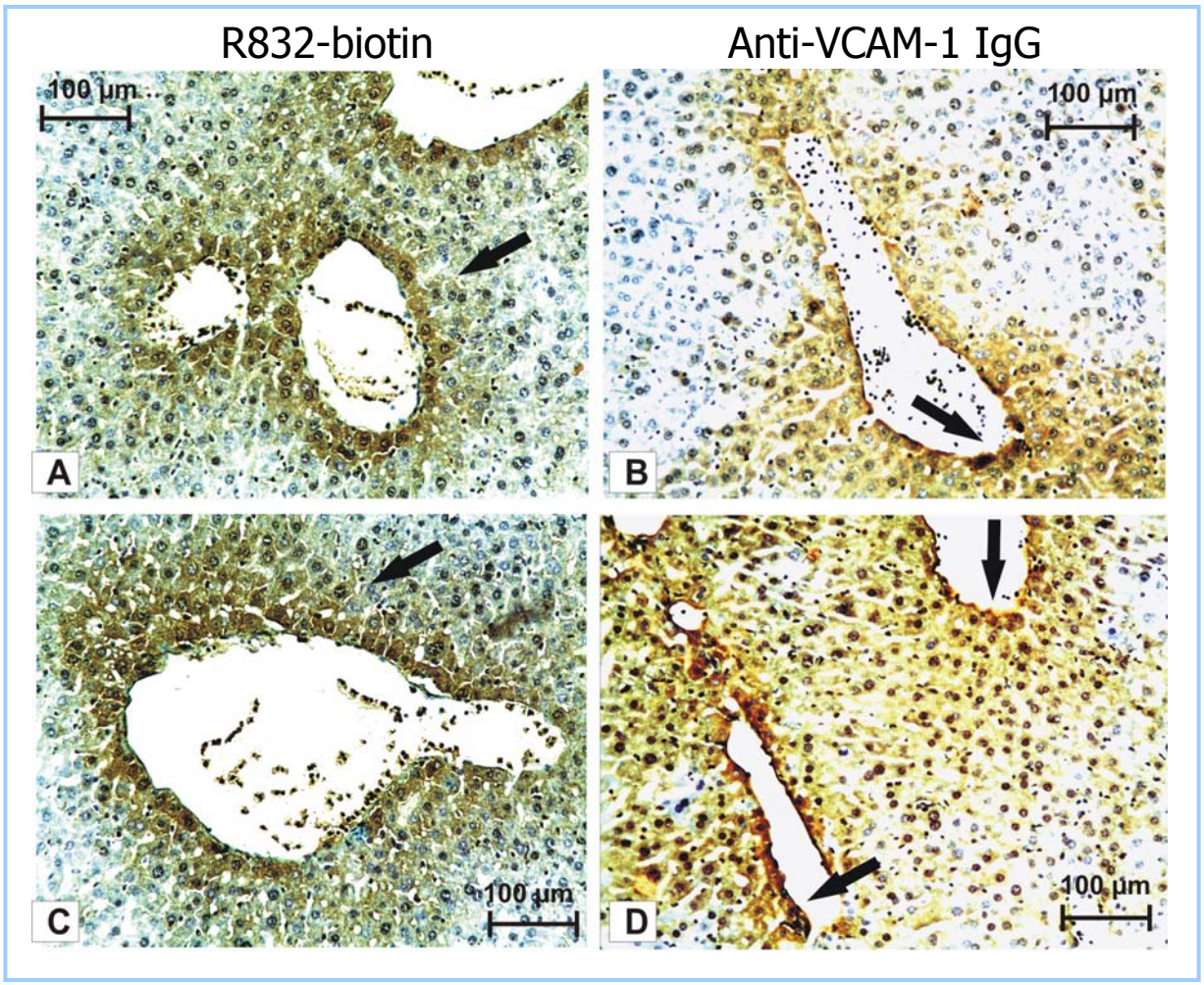


Healthy Gd-DOTA-R832



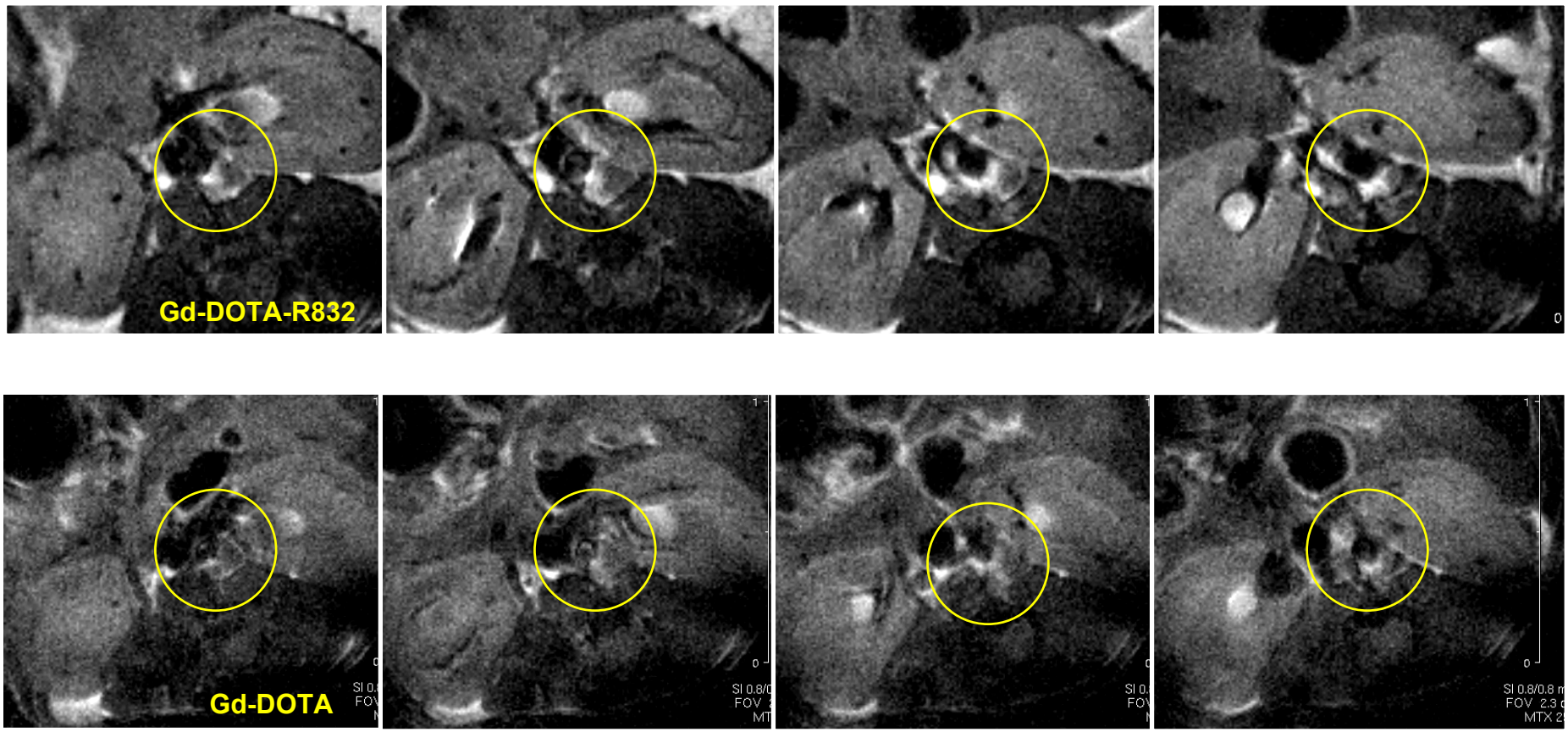
Hepatitis Gd-DOTA

Immunostaining of VCAM-1 expression in liver (mice, concanavalin A) by using biotinylated peptide R832 (A and C) or anti-VCAM-1 antibody (B and D) (stained in brown; arrows)



Molecular MRI of VCAM-1 expression in atherosclerotic plaque (mouse ApoE$^{-/-}$) with Gd-DOTA-R832 (~27 min post-contrast)

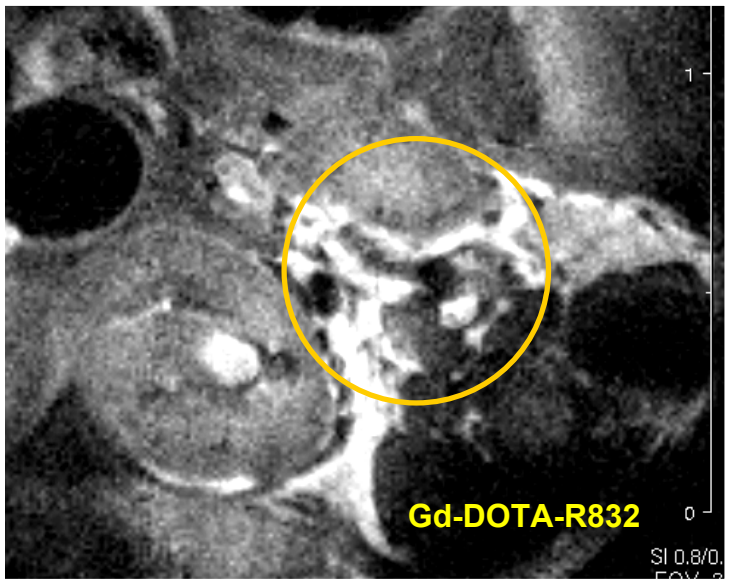
Comparaison with Gd-DOTA (Successive slices of aorta on a length of 3.2 mm; Avance-200 MRI, 4.7 T, RARE, TR/TE = 1048.5/4 ms, RARE factor = 4, FOV = 2.3 cm, slice thickness = 0.8 mm, matrix = 256, NEX = 4, TA = 5'14" spatial resolution = 90 µm)

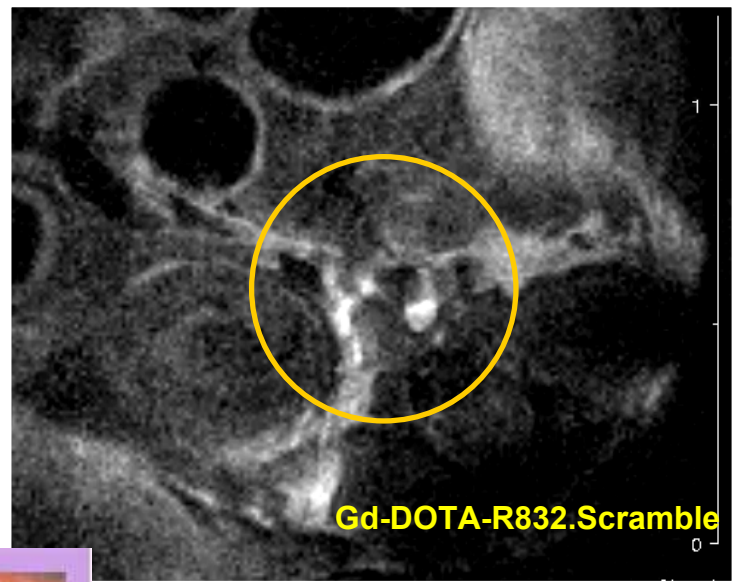


Molecular MRI of VCAM-1 expression in atherosclerotic plaque (mouse ApoE$^{-/-}$) with Gd-DOTA-R832 (~27 min post-contrast)

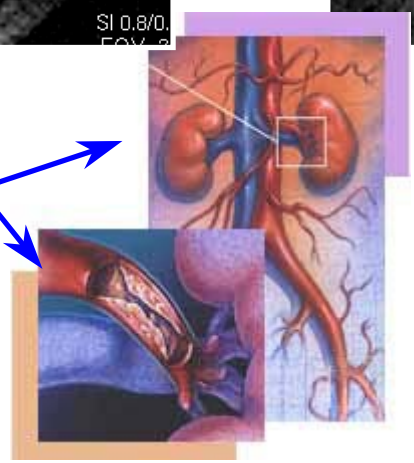
Comparison with Gd-DOTA-R832.Scramble

(Avance-200 MRI, 4.7 T, RARE, TR/TE = 1048.5 / 4 ms, RARE factor = 4, FOV = 2.3 cm, slice thickness = 0.8 mm, matrix = 256, NEX = 4, TA = 5'14" spatial resolution = 90 µm)

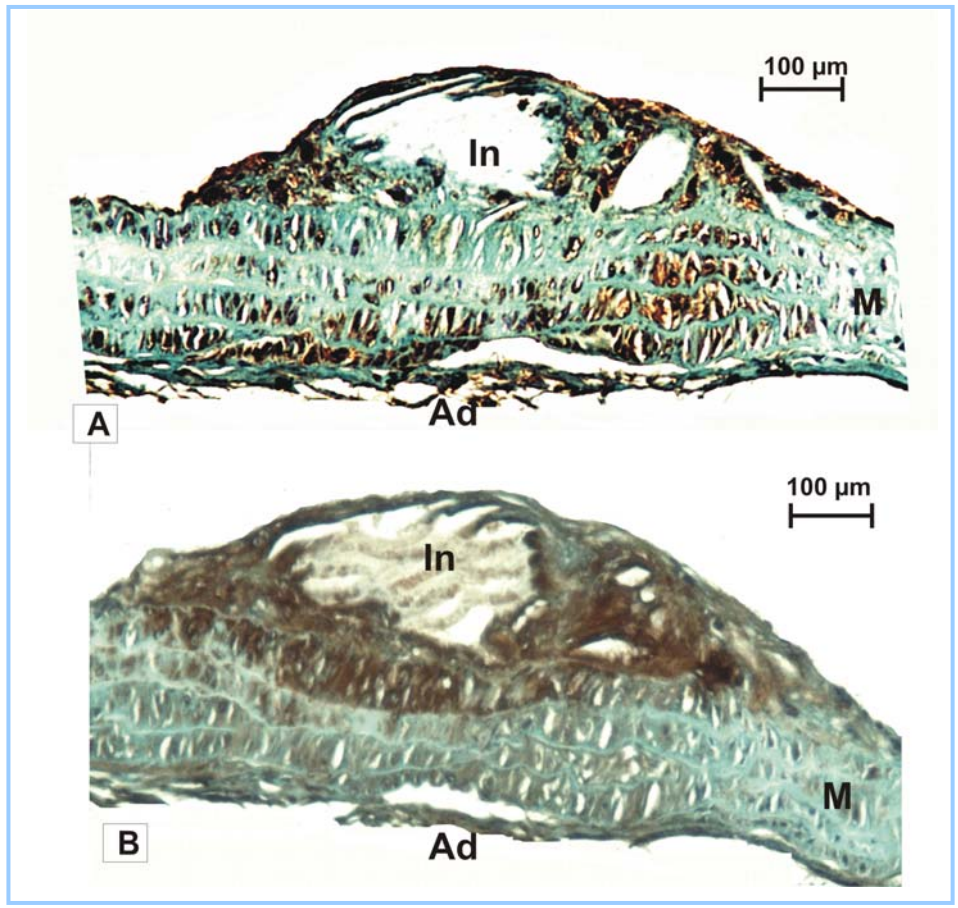




Stenosis of renal artery



Immunostaining of VCAM-1 expression (ApoE$^{-/-}$ mice) by using biotinylated peptide R832 (A) or anti-VCAM-1 antibody (B) (stained in brown)



In = intima; M = media; Ad = adventitia

Conclusions

PEPTIDE R832:

- Important specific affinity for VCAM-1 (purified or expressed by HUVEC)
- Able to identify VCAM-1 expression *in vivo* in pathological models
- Colocalisation of VCAM-1 and binding of R832-biotin
- Therapy: possible anti-inflammatory therapy