**INTRODUCTION**

Widely used in therapeutic research, phage display technology has been implemented in our laboratory to design MR contrast agent (CA) for molecular imaging\(^1\). Phage display allows the identification of high affinity binding peptide (i.e. for a molecule diagnostically relevant) from a heterogeneous mixture of bacteriophages called random library. These phages are different from each others by the sequence of the oligopeptide carried by a protein of the phage wall in such way that all the possible peptide sequences are statistically represented. The sequence of the peptide is determined by sequencing the genome of the corresponding binding phage isolated after several rounds of affinity selection. The MR CA is finally obtained by coupling the selected peptide to a magnetic center. In previous works\(^1\), we have developed the concept of magnetophages, entities obtained by directly coupling USPIO to phages isolated after the phage display procedure. We have shown that magnetophages can be used as in vitro MR contrast agent. In this study, we evaluated the possibility to extend in vivo the use of magnetophages. Nevertheless, the major problem is their non specific phagocytosis by the cells of the reticulo-endothelial system (RES), mainly the Kupffer cells. Consequently, magnetophages are rapidly accumulated into the liver, making them unavailable in the blood pool to reach its target. This problem was abrogated by surface modifications of USPIO by PEG, designing stealthy magnetophages characterized by a much longer circulation time. For this purpose, we have chosen a phage (called E3\(^1\)) specific for phosphatidylserine (PS), a marker of apoptosis\(^2\), as prototype. It will be referred in this paper as magnetophase E3 if magnetically labeled.

**METHODS**

**Synthesis of magnetophages** : Magnetophages were obtained by reaction of the dextran coating of USPIOs with epichlorhydrin and then with phages. Magnetically labeled phages were isolated by selective precipitation with PEG/NaCl. Stealthy magnetophages were obtained by the same way but using pegylated USPIO.

**Saturation and inhibition curves** : Magnetophages (stealthy or not) would be superfluous if the linking of USPIO to the phage wall did not alter the interaction properties of the corresponding non-magnetic phages. The affinity of magnetophages E3 was tested by comparing their Kd towards PS to the Kd of the corresponding non-magnetic phages. This evaluation was performed by ELISA and by competition with annexin V, the natural binder for PS (figure 2), the binding of magnetophages E3 to PS decreases with the increase of the competitor, indicating that magnetophages keep their specifivity for the target.

**In vivo studies** : Stealthy magnetophages and magnetophages, specific or not of the apoptotic marker phosphatidylserine, were injected via the tail vein of anaesthetized male mice bearing apoptotic liver or not. Apoptosis was induced by intraperitoneal injection of 10 µg of anti-Fas antibody. MRI images were acquired using a T2-weighted spin-echo sequence (TR/TE = 2000/20 msec, NN = 4, matrix 128x128, slice thickness = 2.5 mm, FOV = 6 cm) and intensities measured in regions of interest defined in the liver. Analysis of the images is expressed on the modifications of the signal compared to the pre-contrast image and intensities were expressed as relative enhancement in percentage (RE%). Images were acquired on a 4.7 Tesla Avance 200 system (Bruker, Karlsruhe, Germany) equipped with a vertical magnet.

**RESULTS AND DISCUSSION**

Fixation curves of magnetophages E3 and corresponding phages (bearing the same peptide) are shown in figure 1. As seen from these fixation curves, they have an almost equivalent affinity for PS. Kd = 6.2 x 10\(^{-12}\) M and 1.5 x 10\(^{-13}\) M, respectively for specific phages and magnetophages.

Figure 1 : Fixation curves of phages and magnetophages bearing a PS specific peptide.

In the competition experiment with annexin V, the natural binder for PS (figure 2), the binding of magnetophages E3 to PS decreases with the increase of the competitor, indicating that magnetophages keep their specifivity for the target.

**REFERENCES**