



# Use of PEPscreen® Libraries to Identify CD8 Epitopes Presented During Malaria Infection

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Malaria caused by *Plasmodium* species kills more than one million people per year, 90% of them in sub-Saharan Africa. An understanding of immunity to this disease can contribute to the development of vaccines, which would be invaluable in eradicating this disease. Our previous work had shown that CD8 T cells are a major component of immunity to the first stage of infection, that is, the infection of liver hepatocytes following the inoculation of *Plasmodium* sporozoites into the bloodstream of the individual (Morrot et al., 2005). Many of our previous studies have been done using a cell line and T cells from a transgenic mouse specific for an epitope in the CSP protein, which is highly expressed in sporozoites (Morrot and Zavala, 2004). However, this epitope is specific for the MHC molecule Kd found in BALB/c mice. In order to expand our experimental systems and take advantage of the availability of numerous knockout C57/B6 mice, our goal is to identify a sporozoite epitope that is presented by the MHC molecules Kb and Db found on this background.

We have taken advantage of the sequencing of the rodent malaria *Plasmodium yoelii* and the associated expression data to choose candidate genes which are highly expressed in the sporozoite stage of its life cycle (Kaiser et al, 2004). In addition, epitope prediction algorithms such as SYFPEITHI and BIMAS (Parker et al., 1994; Rammensee et al., 1999) allow us to predict the likelihood of a series of 8-10 mer peptides binding to a given MHC molecule. The high scoring peptides sequences were then ordered from Sigma on a PEPscreen format.

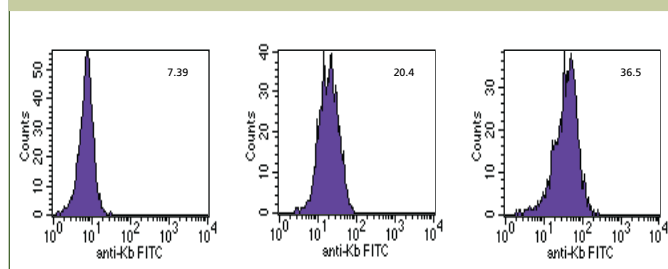
The binding ability of the candidate peptides was screened using the TAP-mutant RMA-S cell line, which is unable to present endogenous peptides. MHC class I expression at the cell surface will be transient unless tight binding peptides are provided in the medium (Rock et al., 1991). Therefore, the cells were incubated with the individual peptides and the surface expression of MHC class I molecules were measured by flow cytometry. The results are shown in **Figure 1**.

The RMA-S assay, though rapid, only indicates that a peptide binds to a Kb or Db MHC class molecule. It does not provide information if a peptide is expressed, processed and presented in a natural infection. To address this issue the ELISPOT assay (Carvalho et al., 2001) was used. Mice were immunized with  $\gamma$ -irradiated sporozoites either once or twice

at 3-week intervals. Five to 10 days after immunization the spleens were harvested. Spleen cells were aliquoted into a 96-well filter plate that had been pre-coated with anti-IFN- $\gamma$  capture antibody. The spleen cells were then stimulated with RMA-S cells pulsed with the peptide. After 24 hours the plate was washed and the IFN- $\gamma$  detected using a detection antibody and standard peroxidase development reagents. The presence of IFN- $\gamma$  was seen as spots on the plate corresponding to cells that release the cytokine in an antigen specific manner.

Future studies will involve making T cell lines for possible *Plasmodium* epitopes identified and studying if such cell lines confer protection against malaria infection and using the lines to analyze how immune responses develop. If this yields interesting results the relevant T cell receptor may be cloned and a transgenic mouse specific for the peptide of interest may be generated.

**Figure 1. Surface Expression of MHC Class I Molecules**



**Figure 1.** Flow cytometry plots of MHC class I (Kb) expression on RMA-S cells following 3-hour incubation at 37 °C without peptide (left), with the previously defined epitope, SIINFEKL (center), and with the novel epitope, STYKKNYPLL (right). Values are mean fluorescent intensity.

## References

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