# 4th Annual Research Symposium
## HFMG Academic Affairs
### (To be held on April 13, 2007 at Henry Ford Hospital, E&R Building)
# Abstract Deadline: March 23, 2007

**Immunomodulatory characteristics of magnetically labeled macrophage like THP-1 cells**

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**Introduction:** Previously, we have reported a novel method for generating magnetically labeled cells that is based on combining ferumoxides and protamine sulfate (**FePro;** both FDA approved agents) into Superparamagnetic Iron Oxide (SPIO)-transfection agent complex. Magnetically labeled cells can then be used as an *in vivo* magnetic resonance imaging (MRI) probes for localizing and monitoring physiological/ and or pathological processes. However, certain number of locally or systemically administered magnetically labeled cells may be cleared from the tissues by host macrophages. For successful clinical application of SPIO labeling method it is important that this mode of *in vivo* clearance of iron does not elicit any diverse immunological effects. In this study we used THP-1 cell line that has widely been used as a model for studying macrophage cell type, to demonstrate that FePro incorporation into macrophages does not alter immunological properties of these cells. We hypothesize that FePro labeling will not affect the physiological ability of THP-1 cells to maintain non-differentiated state nor it will affect their ability to respond to the activation stimuli.

**Methods:** THP-1 cells were magnetically labeled with FePro and the mean intracellular iron was determined by UV/VIS spectrophotometric method using hydrochloric acid and potassium ferrocyanide. After the labeling, cells were differentiated in the presence of 100nM of TPA for 24h. Finally, cells were stimulated with 100 ng/ml of LPS for 30 minutes or 4 hours. After 30 min of LPS stimulation, cells were harvested, cytoplasmic and nuclear protein fractions separated and analyzed by immunobloting for the activation of NfKB/IkB cell signaling pathway. For hours after incubation with LPS, cells were analyzed for the expression of cell surface proteins using specific fluorochrome conjugated antibodies against HLA-DR, CD117, CD54, CD11b and CD83.

**Results:** Western blot analysis of nuclear and cytoplasmic THP-1 cellular fraction demonstrated no difference in the LPS-induced activation of NfKB/IkB pathway between FePro labeled and non-labeled (control) THP-1 cells. In addition, FePro labeling alone, in the absence of LPS stimulation did not result in NfKB/IkB activation. Flow cytometric analysis revealed no differences between FePro labeled and non-labeled THP-1 cell for the levels of TPA- induced upregulation of HLA-DR, CD117, CD54, CD11b and CD83. In addition, the levels of LPS induced upregulation of CD54 and CD83 were comparable in labeled and non-labeled cells, as well.

**Conclusions:** Ferumoxides-protamine sulfate complex does not alter the immunological properties of THP-1 cells. When applied as a labeling agent for various types of cells, labeled cells can be used as *in vivo* probes that can be tracked by MRI imaging. It is possible that this method can be used in various clinical settings without causing any adverse effects that may stem from immunological reaction to the iron clearance by host's macrophages.