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**THE REGULATION OF TAP GENE EXPRESSION IN
MACROPHAGES**


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**Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in the Department of Microbiology and Immunology**


Indiana University

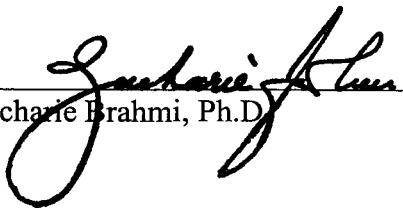
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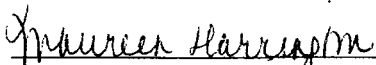
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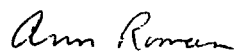
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ABSTRACT

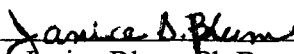
Antigen presentation in the context of MHC class I antigens requires the transport of peptides from the cytosol to the endoplasmic reticulum by the transporter associated with antigen presentation (TAP). The transporter consists of a heterodimer composed of the Tap-1 and Tap-2 proteins. With recent studies suggesting a key role for professional antigen presenting cells in the induction of cytolytic T cell responses, we initiated studies on the regulation of TAP gene expression in macrophages. The two main objectives of this thesis are: 1) to identify how TAP gene expression is regulated in the human macrophage cell line, THP-1 following activation with IFN- γ and/or LPS, 2) to identify cis-elements and trans-acting factors involved in the transcriptional regulation of the Tap-1 gene. Northern blot analysis, half-life and nuclear run-on analysis determined TAP expression is regulated at the level of transcription following stimulation with IFN- γ . No effect was observed on THP-1 cells following stimulation with LPS alone. Treatment of cells with both IFN- γ and LPS altered the kinetics and amount of Tap-1 and Tap-2 expression, as compared to stimulation with IFN- γ alone. Western blot analysis determined Tap-1 protein levels also increased following stimulation. Studies on the Tap-1 promoter indicated that a GAS element is responsible for upregulation following stimulation with IFN- γ alone and IFN- γ plus LPS. Gel shift and supershift analyses indicated roles for the transcription factors Stat 1 α and IRF-1 following stimulation of this macrophage cell line. Co-transfections using expression vectors containing the HCMV IE genes dramatically increased Tap-1 reporter gene activity in unstimulated as well as IFN- γ stimulated cells. This transactivation was promoter specific. Using 5' nested deletions of the Tap-1 promoter, it was determined that the HCMV IE genes

required the ISRE and GAS region for transactivation.

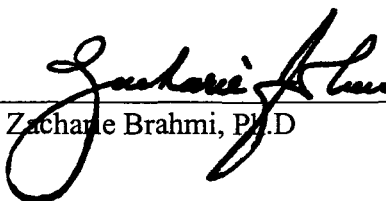


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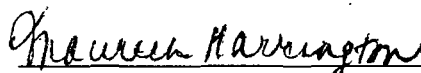
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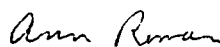


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