SUMMARY

The effects of cAMP elevating agents and glucocorticoid on the production of inflammatory mediators like nitric oxide, TNF-α, IL-12 and anti inflammatory mediator IL-10 were studied in murine peritoneal macrophages. cAMP elevating drugs like theophylline or cAMP elevating agents, Bt_2cAMP and PGE_2 either alone did not show any effect on nitric oxide production but along with IFN-γ and LPS it could slightly enhance nitric oxide production. Dexameathasone, a glucocorticoid, could inhibit NO, TNF-α production in IFN-γ and LPS treated cells but when cAMP elevating agents were added in dexamethasone pretreated and IFN-γ and LPS treated cells, levels of TNF-α, IL-1α, IL-6 was synergistically down regulated but NO production inhibited by dexamethasone was interfered by the presence of cAMP elevating agents which was found to be true at mRNA level as well as at protein level for iNOS without affecting mRNA stability. Dexamethasone did not induce the IL-10 production in IFN-γ and LPS stimulated cells alone but in combination with Bt_2 cAMP produces high amount of IL-10. IFN-γ and LPS stimulated cells produce high amount of IL-12. This production was decreased when Bt_2 cAMP was added in IFN-γ and LPS stimulated cells and was further decreased when dexamethasone and Bt_2 cAMP treatment was given to IFN-γ and LPS stimulated cells. NFκB binding activity down regulated by glucocorticoid was enhanced by the addition of Bt_2 cAMP without inhibiting NFκB protein expression and may be responsible for the upregulation of iNOS expression. Our

results indicate that use of glucorticoids alongwith cAMP elevating agents may be beneficial in lowering the level of inflammatory mediator (IL-12, IL-1α, IL-6) during inflammation by enhancing IL-10 level. On the other hand our results indicated that coincubation of dexamethasone with Bt_2cAMP failed to show synergistic effect on NO inhibition. Therefore, during combined drug therapy by glucocorticoids and cAMP elevating agents may give adverse effects due to cAMP mediated interference of NO inhibition.

Mycobacterium microti infected mouse peritoneal macrophages produce high amount of prostaglandin E_2 (PGE_2) and nitric oxide (NO) when activated with interferon-γ (IFN-γ). In order to understand the relation between PGE_2 and NO production with the induction of immunoregulatory cytokines (IL-12 and TNF-α), macrophages deactivating molecules (IL-10 and PGE_2 and MHC class-II (Ia) molecules by *M. microti* infected and IFN-γ stimulated macrophages, we analysed the level of these molecules in the presence or absence of PGE_2 and NO inhibitors. Addition of N^G-methyl-L-arginine (L-NMMA, 0.5 mM) induced a significant increase in TNF-α (2.1 fold) as well as IL-12 (p40) level (2.6 fold) in comparison to *M. microti* - infected and IFN-γ treated cells. Indomethacin treatment results in increased TNF-α (1.7 fold) as well as IL-12 (p40) level (1.9 fold). On the other hand, the levels of macrophages deactivating molecules (PGE_2 and IL-10) were decreased by 33 and 88 % respectively and indomethacin caused decrease of PGE_2 and IL-10 by 98 and 56% respectively. These results indicate that endogenous TNF-α might be playing a role in growth inhibition of *M microti*. This result was

further confirmed by using anti-mouse TNF-α. Addition of anti-mouse TNF-α along with IFN-γ in infected cells in the presence of L-NMMA was observed to increase bacterial load within the macrophages, suggesting the involvement of TNF-α in killing of mycobacterium.

Enhanced PGE_2 and NO also regulate the expression of MHC class-II (Ia) molecules. MHC class-II (Ia) expression was impaired in *M. microti* infected and IFN-γ treated mouse peritoneal macrophages. Furthermore, down regulation of MHC class-II (Ia) molecules may be due to reduction in tyrosine phosphorylation of substrate proteins. required for IFN-γ signaling.We also found that *M.microti* is sensitive to NO when directly exposed to NO generating compounds but we never got 100% killing by these NO generating compounds. Thus it can be concluded that NO has little effect on the growth inhibition of intracellular pathogen rather it modulates immune response by lowering the production of Th1 activating cytokines IL-12, and increasing the level of immunosuppressive cytokine IL-10 for its survival within host cells. Combination drug therapy with corticosteroid and cAMP elevating agents may have paradoxial effects in tuberculosis patients.