Genome-wide transcription analysis of interaction between the human macrophage and *Mycobacterium tuberculosis* during concurrent drug administration by conventional and novel methods

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**Objective**

- To examine the genome wide expression profile of differentiated THP-1 cells infected with *Mycobacterium tuberculosis* (MtB) upon treatment with free drugs and microparticles.
- To examine whether microparticle phagocytosis alters activation state of infected macrophages and thereby contributes to bacillary load of microparticles.

**Introduction**

- In pulmonary TB, inhaled bacteria colonize and proliferate within AMs, modulating M6 functions to their own advantage. 
- Targeting MP directly to AMs via inhalation therapy improves efficacy of existing drugs. (Sen et al, PCT'07
- High efficacy of MP is not explainable simply on the grounds of enhanced drug delivery, in the light of experience with high-dose chemotherapy.
- Phagocytosis of foreign particles activates several M6 responses, including induction of apoptosis.
- MP induce oxidative radicals and Th1 cytokines TNF-α and IL-12 in infected M6s. (Sharma et al, JAC 2007)
- MP induces apoptosis in THP-1 cells infected with H37Rv which reduces bacterial load. (Yadav & Misra, AAC 2007)
- Apoptosis, rather than M6b-induced necrosis, is a legitimate host-defense strategy.
- We examined the cytokine profiles & gene expression profiles of macrophages infected with Mtb H37Rv after treatments with drugs in solution, drug containing MP or blank microparticles.
- We examined apoptosis induction in the presence or absence of caspase-3 inhibitor, caspase-3, 8 & 9 activity in cell lysate & nitric oxide induction in infected macrophage treated with drugs in solution, microparticles or blank microparticles.

**Experimental**

- **Differential THP-1**
  - Infected with H37Ra or H37Rv

**RNA (0, 12 & 24 hrs): Affymetrix HG U133 Plus 2.0 arrays; labeling, hybridization, scanning and data analysis at TCGA, New Delhi.

**Results**

- Induction with H37Ra or H37Rv
  - Treatment
  - Cell lysate: caspases by fluorescence assays; CFU.
  - Supernatant: cytokines by CBA (BD); NO using Greiss' Reagent

**Conclusions**

MP treatment induces maximum TNF-α, IFN-γ & IL-10 in infected cells. MP induce maximum apoptosis compared to other treatments in infected cells. Apoptosis induction was caspase dependent. Apoptosis induction in all groups was caspase dependent except in Blank MP treated group. MP induce maximum NO in infected or normal cells after treatments in THP-1 cells but in the case of primary macrophage NO induction level varied from donor to donor. This was also true of Th1 cytokines. Bacterial action of microparticles varied from donor to donor in cells from one donor maximum bacterial activity was observed in infected group treated with MP, in another, blank MP were able to reduce bacterial load significantly. Microarray data on at least 2 fold up- or down-regulated genes of different groups with reference to gene expression by uninfected, untreated cells were clustered at different time points. At 0 & 24 hrs uninfected cells treated with microparticles clustered separately from all other groups, suggesting that the macrophage response to microparticles is concluded in 24 hrs in absence of infection. At 12 hrs, gene expression profile of infected cells given any treatment clustered separately from cells given either infection alone or microparticles alone, suggesting that phagocytosis-induced gene expression has more in common than expression modulated by infection subjected to treatment. About 1400-1700 genes were differentially regulated by different treatments. These belonged to families such as cytokines, chemokines, and their receptors, GPCR, transcription factors, pro- and anti-inflammatory, and housekeeping genes.

Pathway analysis of FAS and TNF-mediated apoptosis induction presented here indicates that infection alone downregulates pro-apoptotic pathway, while microparticles tend to either reverse this strategy or actively promote apoptosis induction, e.g. TRADD.

**References**

Sen et al, PCT'07


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