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Microparticles treatment modify Apoptosis responses in differentiated THP-1 cells Infected with *Mycobacterium tuberculosis*

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Objective

To test the hypothesis that microparticles (MP), with or without anti-tubercular drugs, activate a pro-apoptotic response in differentiated THP-1 cells infected with *Mycobacterium tuberculosis (M.tb.)*; in the terms induction of early and late apoptosis, alteration of mitochondrial membrane potential, caspase-3, 8 & 9 activity, P2R activity.

Introduction

- In pulmonary TB, inhaled bacteria colonize and proliferate within AMϕ, modulating Mϕ functions to their own advantage.
- Targeting MP directly to AMϕ via inhalation therapy improves efficacy of existing drugs. (Sen *et al, PCT Int' l Pat App. 20050084455, October 16, 2003*)
- MP induce oxidative radicals and Th1 cytokines TNF-α and IL-12 in infected Mϕ. (Sharma *et al, communicated)*
- We examined the time kinetics of secretion of TNF-α and its role as a possible mediator of a pro-apoptotic response by infected Mϕ to combat infection.
- We examined the induction of caspase-3, 8 & 9, alteration of MMP, P2R activity, induction of early apoptosis and late apoptosis.

Methods

Experiment 1 (*In vitro*)

Cytokine production by the cultured murine Mϕ cell line J774 A.1 as a function of time after infection and treatment

Experiment 2 (*In vitro*)

Early apoptosis induction analysis in differentiated TH P-1 cell line infected with *M tb* H37Ra, stained with annexine V and propidium iodide (PI)

THP-1 cells, (infected, treated, incubated as above)
↓ Spun at 1000 rpm for 5min
Added 1XPBS 200 μl & resuspend
↓ Removed supernatant
Re-suspended in 1X binding buffer
↓
Added 2 μl of FITC conjugated annexine V
↓ Incubated in dark for 15min
Spun at 1000 rpm for 5 min & supernatant removed
↓ Re-suspended in binding buffer
Added 10 μl of PI
↓
Flow cytometry

Experiment 3 (*In vitro*)

Alteration of MMP in differentiated THP-1 cell line infected with *M tb* H37Ra, stained with Rhodamine123 (Rh123)

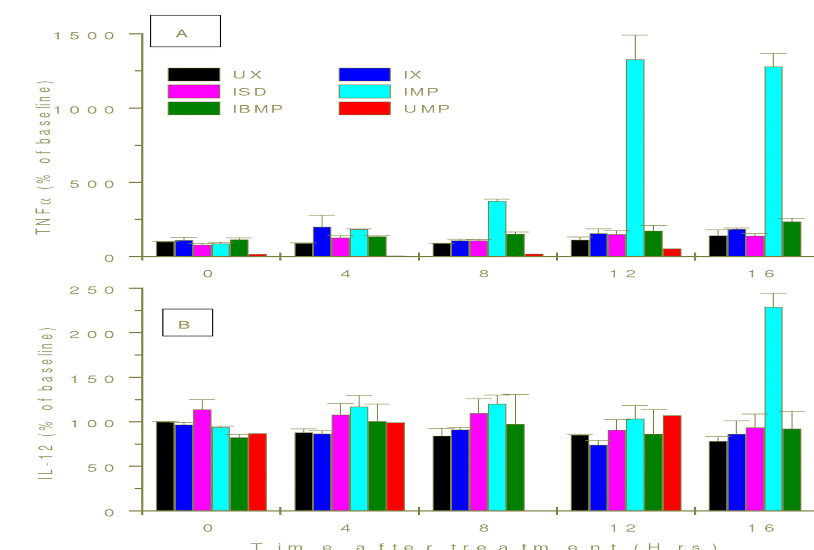
THP-1 cell line (Monocytes)
↓ PMA(20nM/ml medium)
Differentiated THP-1 cells
↓ *M Tb* H37Ra; MOI 20; 2 hrs
Infected cells
↓ Treatment (2hrs); Washing
Incubated 12hrs
↓
Added Rh123 (5mM), incubate 30min.
↓
Washed
↓
Flow cytometry

Experiment 4 (*In vitro*)

Caspase-3, 8 & 9 induction in differentiated THP-1 cells in response to infection and treatment

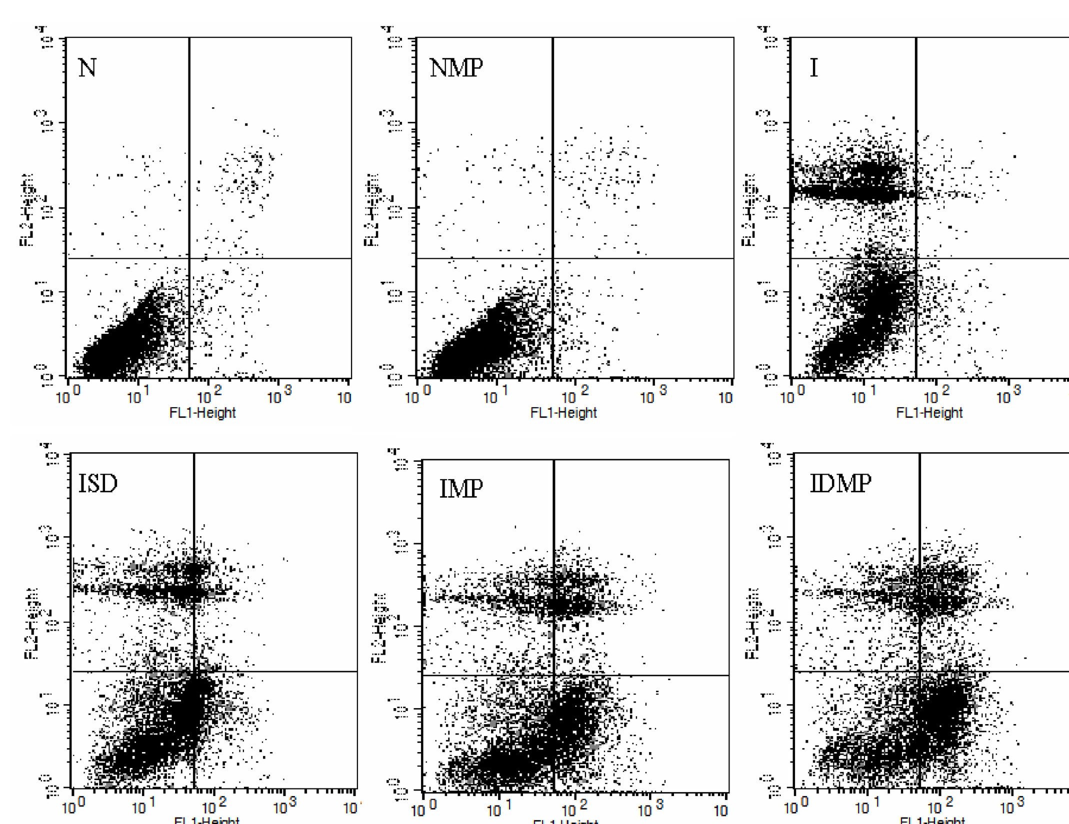
THP-1 cells, (infected, treated, incubated as above)
↓
Lysed (Freeze-thaw in EGTA, EDTA, Tris)
↓
Protein estimated (Bradford reagent, Bio-Rad)
↓
Caspase-3 assay (Lysate containing 10μg protein + equal vol. of assay buffer, incubated 30 min; 37°C).
↓
Added substrate (10 μM coumarin-conjugated peptide)
↓
Fluorescence at λex = 360 and λem= 460

Inhaled MP Induce TNF and IL-12 in Infected J774 A1.



Kinetics of secretion of TNF-α and IL-12 by J 774 A1 cells after infection and treatment with Rifampicin+Isoniazid; either soluble or as MP or blank MP for 2h.

Phospthatidylserine exposure after infection and treatment



Scatter plots y-axis showing PI positive cells and x-axis showing annexin V positive cells. N: Normal controls, NMP: Normal+ treated MP, I: infected cells, ISD: Infected+ treated SD, IMP: infected+ MP, IDMP: infected+ Blank MP

Alteration of MMP after different treatment and P2R activity

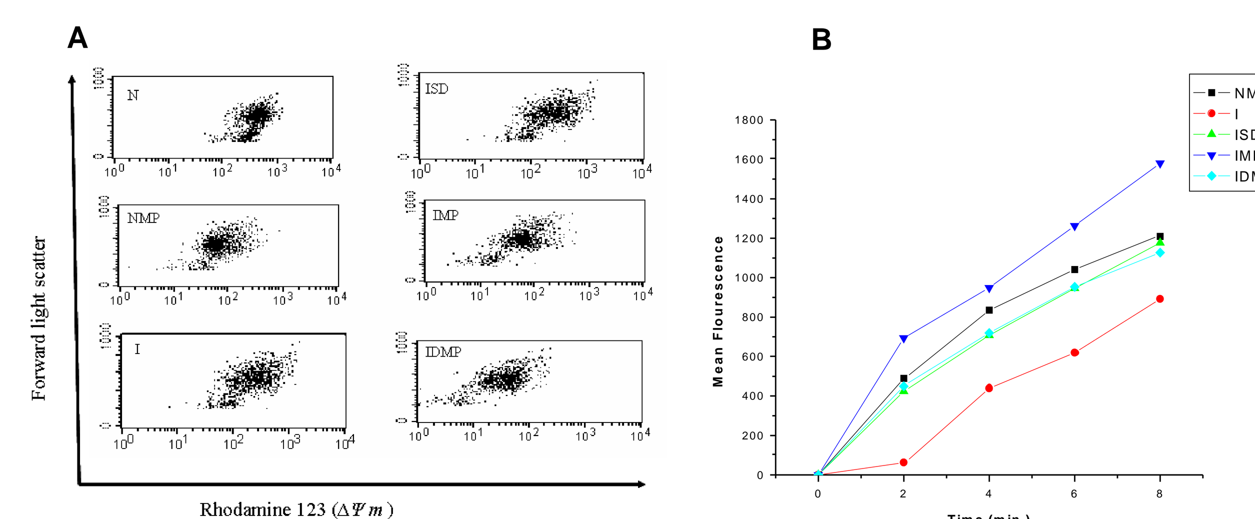
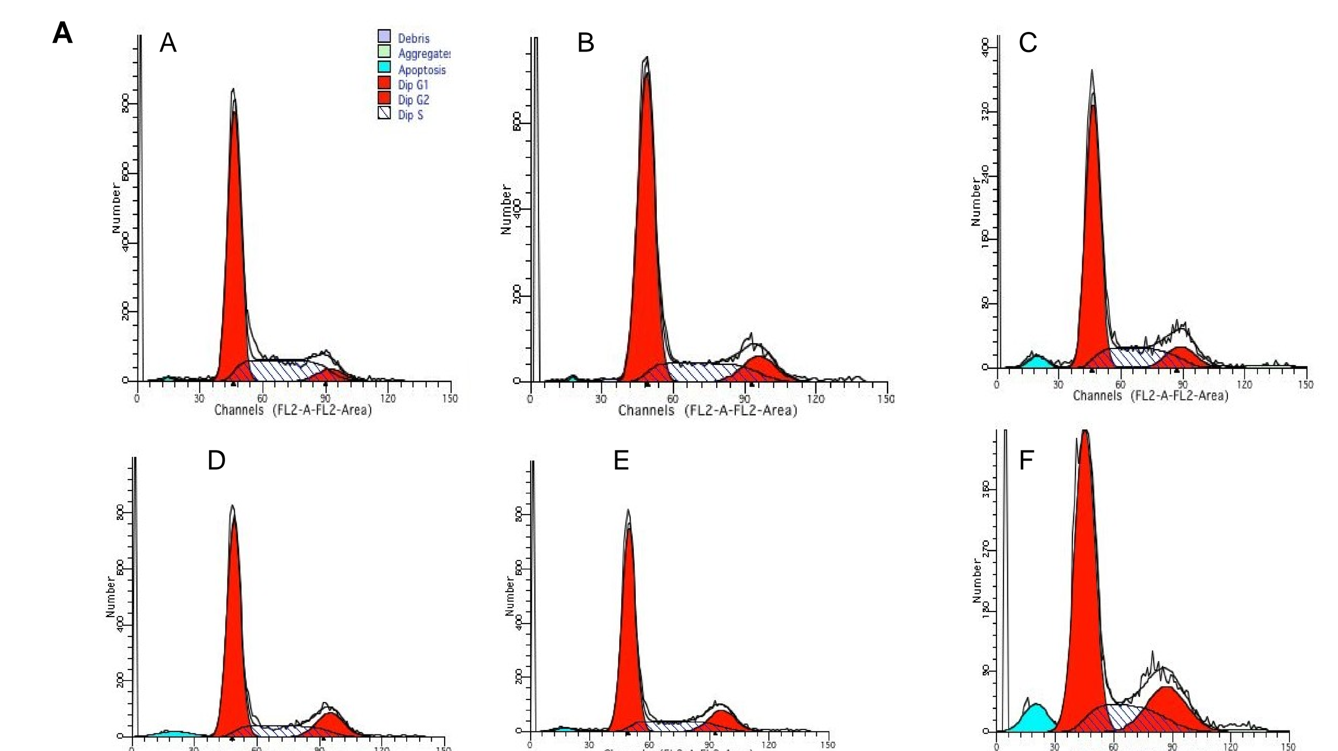


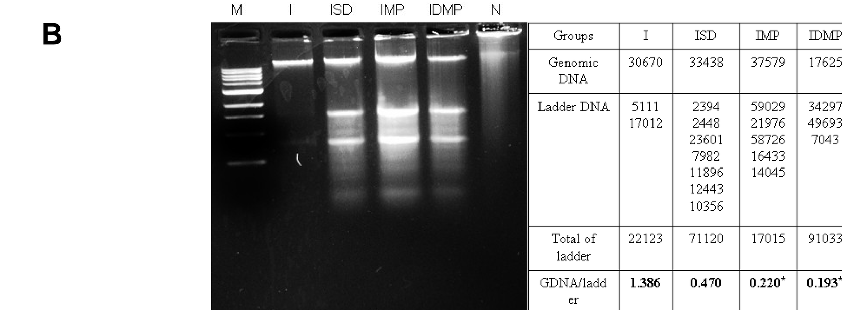
Fig. A: FSC Vs. Mitochondrial membrane potential plotted and in graph B: Mean fluorescence intensity of ethidium influx inside a cells plotted against time after ATP addition

Sub-G1 and G2/M-arrested Cells on MP-Treatment



A: Normal controls, *B*: Normal+MP, *C*: Infected cells, *D*: Infection+Soluble drugs, *E*: Infection+MP, *F*: Infection+Blank MP. Analysed using *Modfit®* after Kamer *et al*, 2005.

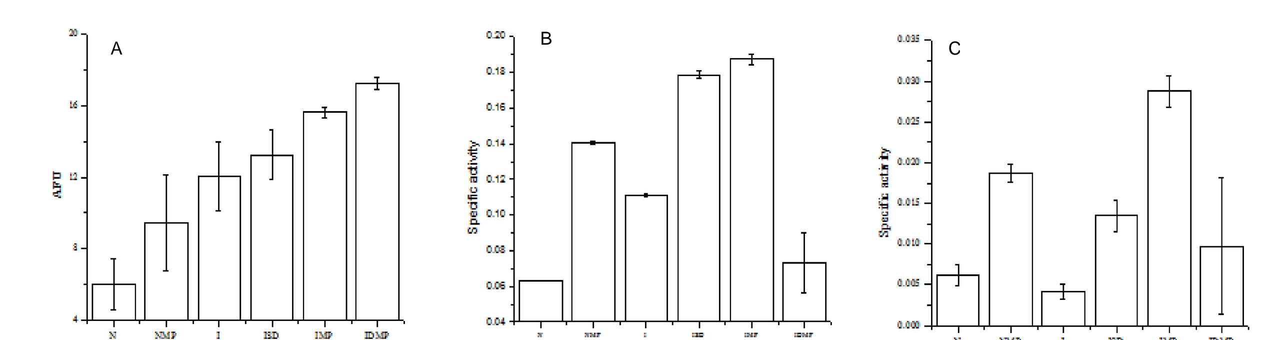
B



Groups	I	ISD	IMP	IDMP
Genomic DNA	30670	33438	37579	17625
Ladder DNA	5111 17012	2394 2448 23601 7982 11896 12443 10356	59029 21976 58726 16433 14045	34297 49693 7043
Total of ladder	22123	71120	17015	91033
GDNA/ladder	**1.386**	**0.470**	**0.220***	**0.193***

M: 1Kb marker, I: Infected cells, ISD: Infected+SD, IMP: Infected+MP, IDMP: Infected+Blank MP)

Different treatment Induces Caspase-3, 8& 9 activities



In graph A. Arbitrary Fluorescence Units (AFU) of Caspase-3 substrate breaking, B. Specific activity of caspase-8 in cell lysate and C. Specific activity of caspase-9 in the cell lysate plotted.

Discussion

We propose that inhaled microparticles evoke a pro-apoptotic response in Mϕ infected with *M tb.* in two ways-

By providing a direct stimulus to the infected Mϕ through the event of phagocytosis.

Indirectly, through the anti-bacterial action of the incorporated drugs, resulting in nullifying the pathogen' s immunosuppressive strategy.

Apoptosis is a valid anti-infective response, since it denies sanctuary to the pathogen which has evolved to survive and replicate within Mϕ .

Further evaluation of the interplay between bactericidal drug action leading to Mϕ survival and induction of innate bactericidal responses, some of which may lead to apoptosis, is in progress.

References

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