

# Preliminary report on immunomodulation of mesenchymal stem cells in M.tb infection

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## Abstract

Tumor necrosis factor-alpha and interferon-gamma are two important pleiotropic inflammatory cytokines that perpetuate tissue damage after increased stress and in the presence of excess glucocorticoids with respect to tuberculosis (TB) infection. Activated macrophage dysfunction leads to granuloma formation, cavities, and fibrosis. Current treatment strategies are not amenable to long-term compliance, contraindications, increased relapse rates, and compounding drug resistance. A natural supplement devoid of the abovementioned complications needs to be developed and checked for healthy immune reconstitution targeting

dendritic cell therapy

. The preliminary prospective study in five TB subjects was assessed objectively using ELISA for TNF-alpha and interferon-gamma levels. The PBMCs were mixed and cultured for the effect of allogenic bone marrow-derived mesenchymal stem cell interactions and secretory cytokines in the culture supernatant. The TNF-alpha levels on day five with an MSC dose of  $10^5$  cells/ml for  $1 \times 10^6$  cells/ml of PBMCs of diseased patients were significantly decreased while there was no change in IFN-gamma levels. The variable dose of MSC appears to have had a significant impact on the cytokine milieu in vitro. The results are encouraging in that there is possibility for effective immune modulation using mesenchymal stem cells in infection-mediated inflammation. Keywords: Mesenchymal therapy; Pulmonary TB infection ; Cytokines ; Immunomodulation ; Mesenchymal stem cell dose.

## INTRODUCTION

### TB AND THE INFLAMMATORY CYTOKINES

Cytokines are soluble, extracellular proteins released by cells that regulate the immune response. Cytokine actions include the generation of inflammation and the activation of phagocytes and B cells. In pulmonary Tb infection, the secretion of the pro-inflammatory cytokines include IL-12, IL-18 and TNF-alpha during early stage of infection, which is essential for induction of Th1 response. TNF-alpha, IL-12, and IL-18 promote clearance of the infection by enhancing the levels of IFN-gamma produced by different cell populations, including T cells, NK cells and gamma-delta T cells. In contrast, early secretion of IL-4, IL-10 has been to polarize the immune response toward Th2 (1,2).

Protective immunity requires a Th1 response, and tumour necrosis factor (TNF-alpha) is needed as an additional, macrophage activating factor (3). With a mixed Th1-Th2 cytokine response, TNF becomes toxic and mediates the gross tissue destruction characteristic of active, progressive tuberculosis (4,5). Stress and corticosteroids tend to drive

newly recruited T cells towards Th2 responses. The protective Th1 immunity, a characteristic feature of granuloma is regulated by two mechanisms i.e., IFN-gamma itself, while essential for granuloma formation, may also limit the uncontrolled expansion of mycobacteria-reactive T cells (3,6,7). Secondly, endogenous IL-10, produced during mycobacterial infections, can reduce IL-12 production from mycobacteria infected DC's, migration of DC's to draining lymph nodes and the expansion of IFN-gamma secreting T-cells. It is to be seen if these dendritic cells play any role in skewing the immune responses and reduce the inflammation, particularly the TNF-alpha and IFN-gamma responses in mycobacterium infection (8).

### MATERIAL & METHOD

The prospective study has IRB and IEC clearance. The subjects recruited into the study were new sputum positive cases without prior treatment for TB. The subjects had proven case history of TB with sputum positivity and X-ray findings and culture proven. Our analysis included data from 5 subjects all of whom were new sputum positive(+3) and

culture proven. The venous blood was drawn prior to the initiation of DOTS treatment.

### **MSC CULTURES**

Bone marrow aspirates were collected from iliac crest in 6%ACD solution after obtaining high risk informed consent. The aspirates were diluted with equal volume of normal saline. Bone marrow mononuclear fraction was obtained using Ficoll gradient. The mononuclear fraction was plated at 25,000 cells in T25 flask. Non-adherent component was removed on day 3 and cultured further with DMEM (Gibco,USA) supplemented with 10% FBS (Gibco, USA), FGF2 at 10ng/ml (Sigma, USA) Streptomycin and Pencillin. The confluent cultures on day 14 were trypsinised and expanded up until Passage 2.

### **ISOLATION OF PBMC'S**

Peripheral blood (5.0 ml) and Plain blood (2.0ml) was drawn from 5 diagnosed TB subjects in heparin vacutainer with informed consent. The mononuclear fraction was obtained the same way as from bonemarrow on ficoll gradient. The PBMC's count was adjusted to  $1.0 \times 10^6$  cells/ml. Serum was separated for cytokine analysis.

### **MIXED LYMPHOCYTE CULTURES**

The PBMC's were cultured at a concentration of  $1.0 \times 10^6$  cells/ml as against three different doses of mesenchymal stem cells i.e.,  $10 \times 10^3$  cells/ml,  $10 \times 10^4$  cells/ml,  $10 \times 10^5$  cells/ml in triplicate. PHA (Sigma, USA) was used as a positive control for PBMC's stimulation. The cultures were incubated at 5% CO<sub>2</sub> for 5 days. The culture supernatants were collected on day 5 for cytokine analysis.

### **CYTOKINE ANALYSIS**

The serum and culture supernatants were processed by ELISA for TNF-alpha and Interferon-Gamma levels. (Invitrogen, USA). Briefly, the serum and the culture supernatants were incubated with respective antibodies followed by rigorous washing and removal of excess non-specific antibodies. The secondary antibody IgG type was conjugated to horseradish peroxidase enzyme and incubated with the antigen-antibody complex. The captured antibody was quantified with the aid of chromogen substrate and readings of the optical density at 450nm. The values were converted to unit measurement using the standard plot.

### **STATISTICAL ANALYSIS**

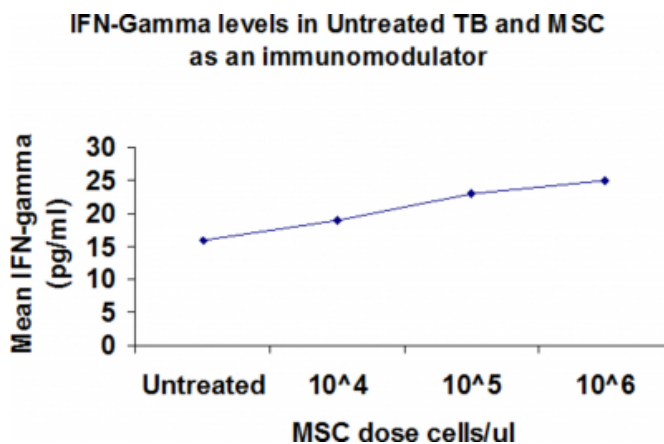
Student's 't' test was performed for statistical significance.

## **RESULTS**

TNF- $\alpha$  and IFN- $\gamma$  levels were gauged in serum from untreated TB cases and compared with cytokine levels obtained from culture supernatants of their peripheral blood MNCs when co-cultured with three different doses ( $10^3$ ,  $10^4$  and  $10^5$  cells/1 $\mu$ l) of MSCs from healthy individuals. Figure 1 demonstrates the TNF- $\alpha$  levels of the 5 subjects prior to treatment and when 1 million MNCs from each of them was treated with three different doses of allogeneic MSCs. The figures demonstrate a peak at  $10^3$  cells/1 $\mu$ l and a significant trough at  $10^5$  cells/1 $\mu$ l of MSC. Figures 2 represents the IFN- $\gamma$  levels obtained when left untreated and their corresponding values when co cultured with variable doses MSCs. The levels of IFN- $\gamma$  increased with an increase in the dosage of MSCs. Also a paired t-test was performed to check if there was any significant difference in the cytokine values of the patients prior to treatment and after treatment with varying doses of MSCs. At concentrations of  $10^4$  and  $10^5$  cells/1 $\mu$ l of MSCs, there was a significant difference observed ( $p=0.20$ , at 80% confidence level,  $t < 0.90$ ) in the TNF- $\alpha$  levels. But no such results were obtained when this test was performed to check the difference in IFN- $\gamma$  levels.

**Figure 2**

Figure.2: IFN-gamma values of the untreated serum Vs. mixed culture supernatants.



{image:2}

## **DISCUSSION**

The present study was conducted with an aim to study the ex vivo cellular interactions between Tb infected individuals when treated with variable doses of mesenchymal stem cells. Pulmonary TB infection is accompanied by the secretion of proinflammatory cytokines like TNF- $\alpha$ , IL-12 and IL-18 which are important for the induction of Th1 response which is involved in protective immunity (9). These promote the

clearance of infection by increasing the levels of IFN- $\gamma$  produced by different cell populations. These cytokines are the ones that bring about reactions like granuloma formation, intramacrophage elimination of the bacillary antigens and fibrosis that leads to the resolving of the disease (6,9,10). But with a mixed Th1-Th2 cytokine response, TNF- $\alpha$  becomes toxic and mediates the gross tissue destruction characteristic of active, progressive tuberculosis(4,5,9).

MSC's have been recently reported to have an anti-inflammatory role in experimental models of acute pulmonary and renal injury(11). MSCs have been shown to exert a profound inhibition on activated T cells when stimulated by allogeneic or mitogenic stimuli(12,13,14). They could somehow skew the immune system towards an anti-inflammatory tolerant phenotype preventing further tissue damage. MSC recruitment would entail the suppression of T cell mediated cytotoxicity which would otherwise lead to necrosis, cavities, a consequence of *M.Tb* infection (10,13,15). The subjects thus selected were all new sputum positive and were not given any treatment. There was a significant difference ( $t < .90$ , 80% confidence level,  $p = .20$ ) between the means of untreated samples and the samples cocultured with  $10^5$  and  $10^4$  cells/1ml of MSC.

At  $10^3$  cells/1ml there was no significant difference. This outcome demonstrates that MSCs invitro could have an impact in skewing a shift in the immune system making it more tolerant and less prone to tissue damage. This is a novel finding and the data generated can be used to conduct an in vivo study. The results of the study are promising and a large studies are warranted to be able to use this natural treatment instead of medication which has side effects. The chief limitation of the present study is to perform in an equal number of normal individuals in comparison with TB subjects, to conclusively report that the MSC's have a critical role in regenerating healthy lung epithelium to resist further damage from infection mediated inflammatory diseases.

## References

1. Fortes A, Pereira K, Antas PR, Franken CL, Dalcolmo M, Ribeiro-Carvalho MM, Cunha KS, Geluk A, Kritski A, Kolk A, Klatser P, Sarno EN, Ottenhoff TH, Sampaio EP. Detection of in vitro interferon-gamma and serum tumour necrosis factor-alpha in multidrug-resistant tuberculosis

patients. *Clin Exp Immunol.* 2005,141(3), 541-548.  
2. Giosue S, Casarini M, Ameglio F, Zangrilli P, Palla M, Altieri AM, Bisetti A. Aerosolized interferon-alpha treatment in patients with multi-drug-resistant pulmonary tuberculosis. *Eur Cytokine Netw.* 2000,11(1), 99-104.  
3. Roach D. R., Bean A. G. D., Demangel C., France M. P., Briscoe, H. and Britton, W. J., TNF regulates chemokine induction essential for cell recruitment, granuloma  
4. *J. Immunol.* 2002, 168, 4620-4627.  
5. Herring AC, Hernandez Y, Huffnagle GB, Toews GB. Role and development of Th1 /Th2 immune responses in the lungs. *Semi Respi Crit Care Med.* 2004; 25: 3-10.  
6. Handzel ZV, Barak V, Altman Y, Bibi H, Lidgi M, Lancovici-Kidon M, Yassky D, Raz M. Increased Th1 and Th2 type cytokine production in patients with active tuberculosis. *Allergy and Clinical Immunology.* 2007; 9: 479-483.  
7. Ehlers S, Benini J, Held HD, Roeck C, Alber G, Uhlig S. Alpha beta T cell receptor-positive cells and Interferon gamma, but not inducible nitric oxide synthase, are critical for granuloma necrosis in a mouse model of mycobacteria-induced pulmonary immunopathology. *J Exp Med* 2001,194,1847-1859.  
8. Demangel C, Bertolino P, Britton WJ. Autocrine IL-10 impairs dendritic cell(DC) derived immune responses to mycobacterial infection by suppressing DC trafficking to draining lymph nodes and local IL-12 production. *Eur J Immunol* 2002,32,994-1002.  
9. Giosue S, Casarini M, Ameglio F, Zangrilli P, Palla M, Altieri AM, Bisetti A. Aerosolized interferon-alpha treatment in patients with multi-drug-resistant pulmonary tuberculosis. *Eur Cytokine Netw.* 2000,11(1), 99-104.  
10. Krampera M, Cosmi L, Angeli R, Pasini A, Liotta F, Andreini A, Santarlasci V, Mazzinghi B, Pizzolo G, Vinante F, Romagnani P, Maggi E, Romagnani S, Annunziato F. Role for interferon-gamma in the immunomodulatory activity of human bone marrow mesenchymal stem cells. *Stem Cells.* 2006 ,24(2),386-98.  
11. Ryan JM, Barry FP, Murphy JM, Mahon BP. Mesenchymal stem cells avoid allogenic rejection. *Journal of inflammation.* 2005, 2,8.  
12. Di Nicola M, Carlo-Stella C, Magni M, Milanese M, Longoni P.D, Matteucci P, Grisanti S, Gianni A.M. "Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli" *Blood.* 2002, 99, 3838-3843.  
13. Agarwal S, Pittenger MF. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood.* 2005, 105,1815-1822.  
14. Sudepta Agarwal, Mark Pittenger F. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood,* 2005,105.  
15. Le Blanc K, Tammik L, Sundberg B, Haynesworth SE, Ringden O. Mesenchymal stem cells inhibit and stimulate mixed lymphocyte cultures and mitogenic responses independently of the major histocompatibility complex. *Scand J Immunol.* 2003;57:11-20.  
16. Klyushchenkova E, Mosca JD, Zernetkina V, Majumdar MK et al. T cell responses to allogeneic human mesenchymal stem cells: immunogenicity, tolerance and suppression. *Journal of biomedical Science.* 2005, 12,47-57.

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