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ANALYZING INTRACELLULAR LEVEL OF IFN- Γ AFTER ANTIGENIC STIMULATION

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ABSRACT:
Interferon-gamma (IFN-γ) is a key cytokine that mediates immunity to tuberculosis (TB). Mycobacterium tuberculosis (M. tb) is known to down regulate the surface expression of IFN-γ receptor (IFN-γR) on macrophages and peripheral blood mononuclear cells (PBMCs) of patients with active TB disease. Many M. tb antigens also down modulate IFN-γR levels in macrophages when compared with healthy controls. In the current study, we aimed investigating the Role of M. Tuberculosis Antigens in Regulating the IFN-γ Receptor Levels in Mouse Macrophages. This down modulation is regulated at the level of TLR signaling pathway, second messengers such as calcium and cellular kinases i.e. PKC and ERK-MAPK, indicating that fine tuning of calcium response is critical to maintaining IFN-γR levels on macrophage surface. In addition, genes in the calcium and cysteine protease pathways which were previously identified by us to play a negative role during M. tb infection also regulated IFN-γR expression. Thus, modulations in IFN-γR levels by utilizing host machinery may be a key immune suppressive strategy adopted by the TB pathogen to ensure its persistence and thwart host defense.
KEYWORDS: Antigens, Macrophages, Antigens, Infection.

INTRODUCTION:

Mycobacterium tuberculosis is a facultative intracellular pathogen that resides and multiplies within human macrophages. M. tuberculosis antigens play important role during course of infection. M. tuberculosis secrets different Antigens during course of infection. Several Antigens found to have protective role for the Bacterial survival and replication inside the host systems. Rv2463 and Rv3416 are the Antigens expressed on day 1 and day 5 respectively by Mycobacterium. The interplay between immune activation and immune evasion during M. tuberculosis (M. tuberculosis) the causative pathogen for tuberculosis depends on a number of factors. One major determinant is a balance in the levels of cytokines and their receptors at sites of immune action that is also regulated at multiple levels. One family of proteins that critically determine this balance is Gamma interferon (IFN-γ), the predominant inducer of macrophage mediated microbicidal functions, has been shown to be required for the prevention of progressive M. tuberculosis infection. M. tuberculosis inhibits gamma interferon (IFN- γ) mediated anti-mycobacterial action by adopting diverse mechanisms. IFN- γ binds to its receptor, IFN- γR, in order to initiate proper signaling. IFN- γR consists of two hetero dimeric subunits, IFN- γ R1 (ligand binding) and IFN- γR2 (signaling subunit). The IFN- γR is expressed on lymphoid cells (such as monocytes/macrophages, T, B, and NK cells) and non lymphoid cells (such as fibroblasts and endothelial cells). We have observed surface expression levels of IFN- γ receptor 1 (IFN- γ R1) in J774 mouse macrophage cell line. To delineate the mechanism by which M. tuberculosis modulates IFN- γR1, in vitro experiments were designed, wherein the role of TLR pathway towards antigen mediated IFN γR1 expression was observed. Although TLRs and the innate immune system are essential for defending the host against microbes, the degree of redundancy and specificity manifested in vivo among different TLR family members is only partially understood.

Despite the overwhelming evidence showing a critical role for TLR mediation of M. tuberculosis recognition in vitro, the in vivo significance of individual TLRs has been more difficult to show consistently. Interestingly, TLR9 but not TLR2 was found to control production of IFN-γ from CD4+ T cells in infected mice. Together, these in vivo studies suggest an important though not absolute role for the TLR pathway in mediating host protection to murine M. tuberculosis infection.

Furthermore, Mycobacterium tuberculosis is one of the most ubiquitous pathogens in the world: estimates roughly one third of the world's population is infected with the bacillus, and it is responsible for 8 to 12 million cases of active tuberculosis each year, and 3 million deaths. There is compelling clinical evidence that, in addition to the innate virulence of the tubercle bacillus itself, the host response to M. tuberculosis plays a major role in determining the clinical manifestations and ultimate outcome of persons who encounter this pathogen. In addition, the natural history of active tuberculosis in the pre-antibiotic era was not uniformly grim. A substantial proportion of patients with active disease eventually recovered without specific therapy. Even today, a small subset of patients with multidrug- resistant tuberculosis for which little effective chemotherapy is available will have apparent clinical recovery. Furthermore, both innate resistance and acquired immunity against tuberculosis seem to exist. The widely used BCG vaccine has at least 50% efficacy in preventing some forms of tuberculosis, and some tuberculin skin-test-positive persons seem protected against developing active tuberculosis despite repeated high level exposure to active cases. Re-infection with M. tuberculosis, which with the use of restriction fragment length polymorphism analysis has been recently demonstrated to occur on occasion in patients with advanced HIV infection, is apparently a rare event in patients with intact immunity. Overall then, a substantial amount of clinical experience indicates that host immunity plays an important role in the host-pathogen interaction occurring in persons exposed to M. tuberculosis. Understanding the components of this host response at a basic level is likely to lead to a better understanding of the pathogenesis of tuberculosis in humans and to result in better and novel approaches to prevention and therapy of this disease, which, among adults, remains the leading single cause of death due to infection in the world. In this study, we will preferentially provide data from in vitro studies involving mouse cell lines. Though certainly animal models have been extraordinarily useful in understanding the pathogenesis of tuberculosis when human studies are unavailable. Animal and human data will be contrasted, as this comparison is most useful to demonstrate the limitations inherent to models of tuberculosis, despite their critical role in developing and testing hypothesis about host immunity.

SCOPE OF THE PROBLEM:

Mycobacterium tuberculosis is one of the most ubiquitous pathogens in the world: estimates roughly one third of the world's population is infected with the bacillus, and it is responsible for 8 to 12 million cases of active tuberculosis each year, and 3 million deaths. There is compelling clinical evidence that, in addition to the innate virulence of the tubercle bacillus itself, the host response to M. tuberculosis plays a major role in determining the clinical manifestations and ultimate outcome of persons who encounter this pathogen. In addition, the natural history of active tuberculosis in the pre-antibiotic era was not uniformly grim. A substantial proportion of patients with active disease eventually recovered without specific therapy. Even today, a small subset of patients with multidrug-resistant tuberculosis for which little effective chemotherapy is available will have apparent clinical recovery. Furthermore, both innate resistance and acquired immunity against tuberculosis seem to exist. The widely used BCG vaccine has at least 50% efficacy in preventing some forms of tuberculosis, and some tuberculin skin-test-positive persons seem protected against developing active tuberculosis despite repeated high level exposure to active cases. Re-infection with M. tuberculosis, which with the use of restriction fragment length polymorphism analysis has been recently demonstrated to occur on occasion in patients with advanced HIV infection, is apparently a rare event in patients with intact immunity. Overall then, a substantial amount of clinical experience indicates that host immunity plays an important role in the host-pathogen interaction occurring in persons exposed to M. tuberculosis. Understanding the components of this host response at a basic level is likely to lead to a better understanding of the pathogenesis of tuberculosis in humans and to result in better and novel approaches to prevention and therapy of this disease, which, among adults, remains the leading single cause of death due to infection in the world.

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PATHOGENESIS OF TUBERCULOSIS:

Based on experimental models, four events are well defined in the pathogenesis of pulmonary TB.

A**) Inhalation of the M. tb.** The early events following inhalation of M.tb involve the engulfment of the bacilli by alveolar macrophages and often their immediate killing by different macrophage bactericidal mechanisms, including the generation of (Expand all abbreviations first time) RNI and ROIs. The efficacy of these mechanisms depends on the intrinsic microbicidal capacity of the alveolar macrophages, the pathogenic characteristics of the inhaled M.tb strain and the inflammatory microenvironment at the site of infection.

B) **Inflammatory cell recruitment**. Bacilli which survive proliferate logarithmically within alveolar macrophages and dendritic cells (DC) and induce the production of immune mediators such as TNF-α, IL-6, IL-12p80, IL-1α, IL-1β, that activate macrophages to induce early bacterial killing [15,16]. IFN-γ is a pro-inflammatory cytokine produced by CD4+ and CD8+ T cells as well as by activated NK cells in response to IL-12 and IL-18 produced by alveolar macrophages and DC. In a local lung inflammatory scenario induced by the proliferation of M. tb, peripheral-inflammatory cells, including monocytes, neutrophiles and DC, are recruited to the lung. DC become activated trough TLRs signaling and monocytes become differentiated to macrophages and become effectors cells that produce microbicidal substances including TNF-α, that contributes to the control of M. tb growth, and granuloma formation.

C) **Control of mycobacterium proliferation.** This phase is characterized by the inhibition of the M.tb proliferation with an efficient cell-cell interaction and the formation of a granuloma. As a result of chronic cytokine stimulation, macrophages differentiate into epithelia cells and become fused giant cells. The architecture of the granuloma is characterized by the aggregation of T cells and infected macrophages which contain the M. tb preventing their spread. In addition to the key role of pro-inflammatory cytokines (eg: IFN-γ, TNF-α, IL-6, IL-12, IL-17 and IL-23) in the formation and stability of the granuloma, the presence of chemokines such as CCL2, CCL3, CCL5, CXCL8, and CXCL10 is crucial for the recruitment of inflammatory cells to form granulomas. These mechanisms allow the development of a localized primary TB infection which eventually may become a stable (also known as a latent) infection. In more than 90 % of the latent infections, the central caseous infectious foci containing live M.

tb is delimited by the granuloma walls. An active cycle of cellular activation and suppression prevents the replication and spreading of the M. tb.

D) **Post-primary tuberculosis.** As a result of the mycobacteria persistence, associated with a failure in the immuno-surveillance system, latent disease may be reactivated, inducing the damage of nearby bronchi and conditioning the spreading of the M.tb to other areas of the lung.

AIM: To analyze intracellular level of IFN-γ after antigenic stimulation as a function of time

METHODS & MATERIALS:

Florescence-tagged Antibodies to IFN- γR, GAPDH and siRNAs against Prkaa2, Stk22a, Snrk, Usp25, Uchl1, Usp9y, Pim2, Senp8, Lgmn, Ctsh, Dcamkl1, TTN were from Santa-Cruz Biotechnologies or Cell signaling or BD Biosciences, San Diego, CA, USA. For FACS experiments antibodies against, IFN- γR, was purchased from Santa-Cruz Biotechnologies.

Recombinant expression of proteins in E. coli

Rv3416 was cloned in pQE31 (Qiagen) vector, whereas Rv2463 was cloned in pET28b (Novagen, Madison, WI) and expressed as His-tagged recombinant proteins in E. coli following standard procedures. The expression of both proteins was observed as inclusion bodies. Proteins expressed as

inclusion bodies were purified by batch method with Nickel affinity column under denaturing conditions with buffers containing urea, as per the manufacturer's instructions (Qiagen). Excess urea was removed by conventional-step dialysis, with reducing concentrations of urea in 10 mM NaH2PO4 buffer (pH 8).

Stimulation of cells

J774 macrophage cells were stimulated with day 1 (RV2463) and day5 (RV3416) antigen along with 15µg each, this concentration

Flow Cytometry

After stimulation cells were scarped and spin at 2000 rpm for 10 mins. Cells were stained for surface levels of IFN-YR, using fluorescein isothiocyanate-tagged antibodies, followed by incubation with streptavidin phycoerythrin, and analyzed by flow cytometry on FACS Calibur (BD Biosciences). The data were plotted using Cell Quest Pro software.

Western blotting

Levels of various molecules were monitored by western blotting as described earlier.9 At the end of incubation, cells were chilled on ice and washed once with ice-cold phosphate-buffered saline and lysed in buffer containing 10mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.9); 10mM KCl; 0.1mM EDTA; 0.1M ethylene glycol tetraacetic acid (EGTA), 0.5% Nonidet P-40 and 2 mg/ml each of aprotinin, leupeptin and pepstatin. The suspension was centrifuged at 13 000 r.p.m. for 2min at 4 1C. The supernatant was designated as the cytoplasmic extract. In all, 20 mg of cytoplasmic extract were then resolved on 10% SDS-PAGE and subsequently transferred onto nitrocellulose membrane (Hybond C pure, Amersham, Arlington Heights, IL, USA). The blots were then probed with antibodies to various molecules followed by HRP-labeled secondary antibodies. Further, a parallel set of samples was run separately on SDS-PAGE and probed for GAPDH as loading control. The blots were later developed by chemiluminescence using the Luminol reagent (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

RESULT & DISCUSSION:

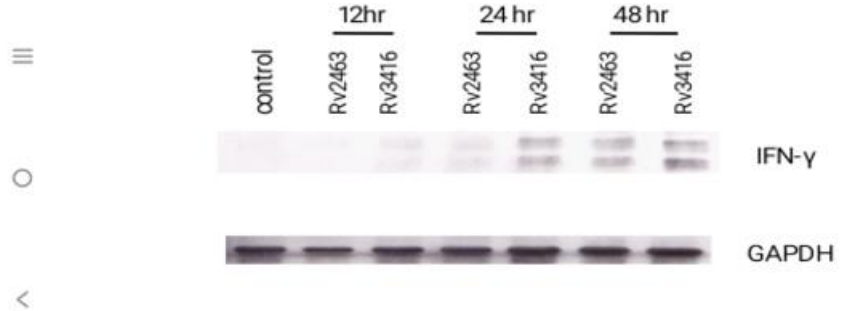

Fig: 1 The up-regulation of Intracellular IFN-gamma by Rv2463 and Rv3416

From this figure we could see that there is up-regulation in intracellular IFN-gamma level upon Rv3416 and Rv2463 stimulation. Interferon gamma (IFN-gamma) is indicated in various cell type (ref...) to prevent cells from entering apoptosis. This result support that M. tuberculosis secretes these antigens in macrophages to create a haven for it survival by increasing the longevity of the macrophage.

IFN gamma though very important for mediating anti-bactericidal responses is thwarted in its effect due to down-regulation of its receptor. It can be seen that Rv2463 and Rv3416, the two potent antigens from M. tuberculosis have the ability to drive suppressive responses in mouse APCs but strangely they upregulate IFN-gamma receptor. Also, in our study we can see it is very well up regulating IFN-gamma receptor level. It’s important here to address the question why M. tuberculosis would try to dig up its own grave. To study whether this up-regulation is through some novel pathway which we can use to potentiate IFN-gamma responsiveness or up-regulation seen was due to some default pathway which got activated while these antigens were mediating some

suppressive responses. For that matter we looked at those pathways which we shown in literature to have some effect on IFN-gamma signaling.

CONCLUSION:

Humans have been infected with Mycobacterium tuberculosis, the disease's causal agent, for thousands of years. M. tuberculosis has the capacity to spread to uninfected people, establish infection, and withstand the host immune response. It must both avoid detection by the host immune system and capitalize on it to finish this infection cycle. An infection with M. tuberculosis frequently results in an equilibrium state marked by bacterial persistence and immune regulation. Recent research has brought to light the several cell groups that are susceptible to M. tuberculosis infection as well as the dynamic shifts that occur in the intracellular and cellular habitats of the pathogen over the course of infection.

REFERENCES:

1. Hess J, Schaible U, Raupach B, and Kaufmann S.H, 2000. Exploiting the immune system: ward new vaccines against intracellular bacteria. Adv Immunol, 1-88.
2. Srivastava V, Vashishta M, Gupta S, Singla R, Singla N, Behera D and Natarajan K, 2011 Suppressors of cytokine signaling inhibit effector T cell responses during Mycobacterium tuberculosis Infection. Immunology and Cell Biology, 1-6
3. Singhal A, Jaiswal A, Arora V, and K. Prasad H, 2007. Modulation of Gamma Interferon Receptor 1 by Mycobacterium tuberculosis, a Potential Immune Response Evasive Mechanism. fection and immunity, 2500-2510.
4. Gupta D, Sharma S, Singhal J. T, Satsangi A. Antony C. and Natarajan K. Apr 12, 2010 Sepression of TLR2-Induced 11-12. Reactive Oxygen Species, and Inducible Nitric Oxide Synthase Expression by Mycobacterium tuberculosis Antigens Expressed inside Macrophages taring the Course of Infection. J Immunol, 5444-5455.
5. William R. Berrington and Thomas R. Hawn, 2007 October. Mycobacterium tuberculosis macrophages, and the innate immune response: does common variation matter? Immunol Rev, 167-186.
6. smghal J, Agrawal N. Vashishta M. GayatriPriya N., K. Tiwari B. Singh Y, Raman R, and rajan K. March 30, 2012 Suppression of Dendritic Cell-mediated Responses by Genes in Calcum and Cysteine Protease Pathways during Micobacterium tuberculosis Infection. The al of biochemistry, 11108-11121.
7. Raviglione, M. C, Snider D. E, Jr, and Kochi A. 1995. Global epidemiology of tuberculosesRidity and mortality of a worldwide epidemic. JAMA., 220-226.
8. Turett. G. S. Telzak E. E. Torian L. V. S. Blum, Alland D, Weisfuse L. and Fazal B. A. 1995. Improved outcomes for patients with multidrug-resistam tuberculosis. Clin Infect. Dis, 1238 1244.
9. Colditz. G. A., Brewer T. F, Berkey C. S, Wilson M. E, Burdick E, Fineberg H. V, and Mosteller F. 1994. Efficacy of BCG vaccine in the prevention of tuberculosis meta-analysis of the published literature. JAMA., 698-702.
10. Stead. W. W, 1995. Management of health care workers after inadvertent exposure to berculosis: a guide for the use of preventive therapy. Ann. Intern. Med., 906-912.
11. Kaufmann S. H, 2001. How can immunology contribute to the control of tuberculosis? Nature Reviews. Immunology, 20-30.
12. Mishra A. K, Driessen N. N, Appelmelk B. J, and Besra G, 2011. Lipoarabinomannan and related glycoconjugates: structure, biogenesis and role in Mycobacterium tuberculosis thysiology and host-pathogen interaction. FEMS Microbiology Reviews, 1126-1157.
13. Kleinnijenhuis, Oosting M, Joosten L. A, Netea M.G. and Crevel R.V, 2011. Innate immune cognition of Mycobacterium tuberculosis. Clinical and Developmental Immunology, 1- 12.
14. Quesniaux V, Fremond C, Jacobs M, Parida S, Nicolle D, Yeremeev V, Bihl F, Erard F, Jania Botha, Drennan M, Soler M.N, Le Bert M, Schnyder B, and Ryffe B, 2004, Toll-like Raptor pathways in the immune responses to mycobacteria. Microbes and Infection, 6946-959.